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BIOLOGICAL CONTROL OF AQUATIC VEGETATION

Thomas T. Sturrock

Florida Atlantic University

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16. Abstract Laboratory studies were conducted to determine optimal growth conditions of Hydrilla verticillata and Eichhornia crassipes on both entire plants and detached leaf cultures. Techniques were then developed for surface sterilization of both the entire and plant parts for both species.

Numerous weed infested habitats in Florida were examined for signs of aquatic plant diseases. These studies indicated various environmental factors as well as possible microbial pathogens as a source of plant tissue debilitation. No significant microbial pathogen was observed for H. verticillata, however, a pathogenic species of a Deutromycete was isolated from a leaf spot on E. crassipes. Studies showed this fungus grows substantially well on common isolation media with the exception of prune agar. Characterization of this fungus revealed it to be an Alternaria species of the order Moniales. It produces hyphae which are both branched and septate, approximately 1.5 nm wide. Conidia are borne on conidiaphores which arise from the hyphae and are typically club-shaped. Infected E. crassipes leaves typically produce leaf spots 14-21 days post-inoculation. Infection was produced via agar block techniques and aerosol spore suspensions. Experiments are described concerning the virulence and host range for this fungal pathogen.

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PREFACE

The Department of Biological Sciences of Florida Atlantic University gratefully acknowledges the following faculty members for their assistance in this investigation: Dr. Manley L. Boss, Professor of Botany, Dr. Walter R. Courtenay, Jr., Professor of Zoology, Dr. Robert B. Grimm, Assistant Professor of Botany, and Dr. Harrison A. Hoffmann, Professor of Microbiology.

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The cooperation of city personnel of many Florida municipalities is greatly appreciated for the assimilation of background data as well as help with collection of plant samples.

This project was conducted under the supervision of Dr. Thomas T. Sturrock, Associate Professor of Botany, as Principal Investigator.

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SECTION I

INTRODUCTION

All plant and animal species, in their native habitats, are subject to natural forces that control their population levels. Natural enemies along with other environmental influences, maintain a natural balance among populations of plants and animals in an ecosystem. There is little question that the parasites, predators, and disease existing in a particular system are the greatest resource that we have for effective pest suppression and management (Train, Busterud & Willard, 1972).

Man steps beyond Nature's boundaries, however, and thereby sidesteps her natural controls by transplanting native plant and animal species to non-native habitats, and in so doing, often causes disastrous shifts in the ecological balance between species.

Such has been the case with many of Florida's noxious aquatic plants. Exotic water plants imported into this country as aquaria specimens have escaped into neighboring lakes and waterways and once established have created serious control dilemmas.

Central and South Florida, like many parts of the United States and the World is in dire need of an efficient and effective means of controlling noxious aquatic weeds. In past years, billions of tax dollars and private funds have been spent on both chemical and mechanical control of these weeds with only semi-effectiveness and even less efficiency. An estimated 10-11 million dollars is being spent per year for the control of aquatic weeds in Florida, and this figure is increasing every year. Despite this huge financial expenditure, the total infestation continues to grow and at present there is no end to the increasing costs unless new control measures are found.

Infamous examples of these aquatic pests include the floating water hyacinth, Eichhornia crassipes (Mart.) Solms, Florida elodea, Hydrilla verticillata (Casp.) and Eurasian water milfoil, Myriophyllum spicatum L.. Other introductions have occurred accidentally, such as alligatorweed, Alternanthera philoxeroides, the result, however, being the same.

Certainly not all aquatic vegetation is harmful to the environment. In fact, quite the contrary; aquatic plants are a necessary part of the water environment, if there is to be fish and other aquatic life. Most of the aquatic plants which are found in Florida waters are not noxious. Those which are classified as such, however, have been without exception imported into Florida.

The rampant growth of exotic water weeds in Florida's water and other southeastern states has been attributed to several factors. First, the average year-round warm temperature and the extended photoperiod combine to give a growing season almost year long. Second, the increased nutrient levels in many bodies of water have been implicated as a major cause of rampant growth (Michewicz, Sutton and Blackburn, 1972), and third, these plants are able to grow and flourish because of the absence of natural enemies normally present in their native environment (Huffaker and Andres, 1970) and lastly, the rapid rate of vegetative reproductior. These four major criteria, along with numerous minor considerations are reasons why some 1,000,000 acres, of Florida's total 2,500,000 acres of fresh water, are infested with noxious aquatic weeds.

For past years, control emphasis has been placed on two basic procedures and only recently a third. Both mechanical and chemical controls have been used routinely in the past and are currently employed for a regular maintenance program. However, neither method on its own is completely satisfactory and at present the weed infestations continue to expand.

The oldest method of weed control is that of mechanical harvesting, which includes every form from hand-picking to sophisticated machinery. Until recently this was the primary technique used in control of noxious aquatic plants. With the advent of chemical control techniques, mechanical harvesting is fading into the background, but there are certain advantages to this method. The first efforts to construct machinery specifically for aquatic control were in 1899 when Congress authorized the construction of two crusher boats, one for use in Florida and one for Louisiana, to be operated by the Corps of Engineers. By 1902 these machines were abandoned because they were inadequate to cope with the problem. A series of more effective and faster operating machines were developed between then and 1937, when handling capacity increased to a 135-ton diesel electric crusher boat. The machinery in use today is more sophisticated and efficient but is still basically a refinement of that developed during the early part of the century.

The primary advantage to harvesting is that the plants, along with the nutrients they absorb, are removed from the water. This tends to reduce the high nutrient levels and prevents buildup of organic material on the bottom of the lake or canal. When plants are allowed to decay in the water, the dissolved oxygen (D.O.) is consumed in the decaying process, and stress on the fish and other aquatic fauna is increased. With mechanical removal, this decay does not occur and the D.O. content is likely to remain at a satisfactory level.

Chemicals were first used to combat water hyacinths as early as 1900. These early attempts were not very successful however.

Raynes reports that 23 different chemicals were tried on aquatics in test runs in 1906. These included arsenite, copper sulfate, formaldehyde, kerosene emulsion and whale-oil-soap solution. Six of these chemicals were found to be effective on aquatics, but all were toxic to cattle and man. As a result, chemicals were not used extensively until the 1940's when 2,4-D was made available (2,4-dichlorophenoxyacetic acid). Since that time, many new herbicides have been developed and put to use in aquatic weed control. Many of the new herbicides are somewhat selective in which aquatic weed they will affect. This does have its advantages in minimizing the effects on the total water environment. In recent years, much controversy has arisen over the effects of placing chemicals in the water and its effect upon the ecology and environment. However at present, herbicides are still the most efficient and effective means of controlling aquatics.

More recently, the concept of biological control has been proposed for aquatic plant pests by numerous investigators. (Zettler and Freeman, 1972) This appears to be one of the most promising tools in aquatic weed control for two reasons:

(1) Only one control application, if successfully established, would be required and

(2) strict pathogens, although often killing individuals in a given population, would not be expected to cause the extermination of a species.

Acceptance of a biological control agent must meet two criteria. First, it must be easily dispersed and have a fairly rapid generation time and second, it must be somewhat specific for the vegetation it is intended, i.e., it must not readily adapt to food sources other than aquatic weeds.

With these as parameters, effective biological control for noxious aquatic weeds have not been fully demonstrated. However, it is hoped that this research project will add additional information to this area of research.

SECTION II

SUMMARY

Laboratory studies were conducted to determine optimal growth conditions of Hydrilla verticillata and Eichhornia crassipes on both entire plants and detached leaf cultures. Techniques were then developed for surface sterilization of both the entire and plant parts for both species.

Numerous weed infested habitats in Florida were examined for signs of aquatic plant diseases. These studies indicated various environmental factors as well as possible microbial pathogens as a source of plant tissue debilitation. No significant microbial pathogen was observed for H. verticillata, however, a pathogenic species of a Deutromycete was isolated from a leaf spot on E. crassipes. Studies showed this fungus grows substantially well on common isolation media with the exception of prune agar. Characterization of this fungus revealed it to be an Alternaria species of the order Moniales. It produces hyphae which are both branched and septate, approximately 1.5 nm wide. Conidia are borne on conidiaphores which arise from the hyphae and are typically club-shaped. Infected E. crassipes leaves typically produce leaf spots 14-21 days post-inoculation. Infection was produced via agar block techniques and aersol spore suspensions. Experiments are described concerning the virulence and host range for this fungal pathogen.

This report was submitted in fulfillment of Project number 801036 under the office of Research and Development, Environmental Protection Agency.

SECTION III

CONCLUSIONS

1. In many instances it is extremely difficult to distinguish between detrimental environmental conditions to aquatic weeds and pathogen damage.

2. Natural damage to aquatic weeds in the field has appeared to be a combination of both environmental parameters and weakly pathogenic or saphrophytic organisms.

3. The *Alternaria* pathogen isolated from *Eichhornia* in Boca Raton, Fla., in its natural physiological state is not a severe enough pathogen to cause destruction of existing water hyacinth populations.

4. Leaf whorl cultures of *Hydrilla verticillata* appear to be a convenient method of screening large numbers of suspect pathogens.

5. Detached leaf culture of *Eichhornia crassipes* proved to be effective in the study of the *Alternaria* pathogen as well as a screening technique for additional suspect pathogens.

6. When preparing plant tissue for pathogen isolation, surface sterilization by means of ethanol flaming or mercuric chloride ($Hg\ CL_2$) rinses proved to be most effective when it was not necessary to keep the host plant viable. When growing host plants in aseptic culture, surface sterilization was best accomplished using a quaternary ammonia compound.

7. Environmental chamber studies showed environmental parameters such as light and nutrient concentrations to cause many of the aberrations and delineation of existing weed populations such as those observed in Bonny Loch and Lake Wales.

8. Under the many varied field conditions, it can be extremely difficult to distinguish between weeds of the genera *Hydrilla Egeria* and *Elodea*.

9. Our survey indicates that at present there is no indigenous microbiological pathogen for either *Hydrilla* or *Eichhornia* in South Florida.

10. The South Florida isolate of *Alternaria* appears to be similar to that species isolated in India.

11. With additional studies, our *Alternaria* isolate has the potential of being a bio-control agent for water hyacinths.

CONCLUSIONS (continued)

12. This species of *Alternaria* shows optimum growth on laboratory media containing sugar additives which is indicative of *Alternaria* being an older leaf disease.

SECTION IV

RECOMMENDATIONS

1. While general surveys for indigenous pathogens should be continued, it is suggested that these efforts be directed towards the plants' native country and habitat.

2. It is suggested that future studies in the realm of biological control of aquatic weeds be encouraged towards the alteration and mutation of existing plant pathogens.

3. It is recommended that studies be undertaken to examine the potential of using biological control in conjunction with other methods such as chemical and/or environmental control.

4. We encourage the use of both the Hydrilla leaf whorl culture and Eichhornia detached leaf culture, as well as in vitro cultivation of plant parts as methods of determining pathogenicity of micro-organisms.

5. The scope of this research did not include viral and rickettsial isolations. Studies in this area are strongly recommended.

SECTION V

LITERATURE REVIEW

One of the largest problems that face aquatic weed research is that of correct identification of the genera Elodea, Egeria and Hydrilla. For years, these three genera have been misunderstood and at present there is still some discrepancy over the taxonomic placement of Hydrilla. Realizing this, we undertook an extensive literature review program which proved to be very beneficial.

The genera Elodea, Egeria and Hydrilla are of the tribe Anachariteae of the family Hydrocharitaceae. The genus Elodea consists of about 15 species, Egeria has 4 species and Hydrilla has only one. This study deals only with Elodea canadensis, Egeria densa and Hydrilla verticillata. The latter two species occur in Florida and live specimens were obtained for examination. All information on Elodea was taken from specimens and descriptions cited in the literature.

As mentioned, one of the several problems in studying these plants is misidentification. Most of the work done in the United States up until 1965 had misidentified Hydrilla verticillata as Elodea canadensis. Previous taxonomists used only vegetative parts of the plants for identification which resulted in the wrong naming of species. The external morphology of all three genera differs with environmental conditions, such as depth, clarity of water, amount of nutrients in water and type of dispersal. One cannot use morphology alone in identification.

Another problem lies in common names which become quite misleading. Some examples are:

American elodea	--	Hydrilla verticillata
Brazilian elodea	--	Egeria densa
Anacharis	--	Egeria densa
Old World elodea	--	Hydrilla verticillata

These plants are receiving more attention in the literature due to their ability to spread fast and create dense infestations. One area which is overlooked in the literature is the life cycles (expecially of Hydrilla) of these plants. More in depth taxonomic studies also are needed. In the past the trend has been to keep these genera separate. However, several taxonomists (Bennett, 1914) feel that they should be condensed into possible one genus.

ELODEA

Elodea canadensis Rich. in Michx., Fl. Bor.-Am. 1:20, 1803; Richard, L.C., Inst. de France, Mem. 12(2):3-4, 1811-12.

Type: Holotype - "in rivulis Canadae." Environs de Montreal, Elodea oblongifolia, A. Michaux (P.)

SYNONYMS:

Anacharis alisinastrum Babington, Ann. & Mag Nat. Hist. II, 1:83-84, 86. 1848;

A. canadensis Planch, Ann. Mag. Nat. Hist. II, 1:86, 1848.
A. canadensis var. latifolia (Casp.) Sanio, Verhandl. Bot. Verein Prov. Brandenburg 32: 121, 1890.
A. canadensis var. latifolia (Casp.) Sanio, var. repens Sanio, Verhandl. Bot. Verein Brandenburg 32: 121, 1890 invalid, a variety under a variety.
A. canadensis var. Planchonii (Casp.) Victorin, Contrib. Lab. Bot., Univ. Montreal 18:40, 1931.
A. Planchonii (Casp.) Rydb., Fl. Prairies & Plains Cent. N. Am. 57, 1932.
A. Planchonii (Casp.) M.E. Peck, Man. Higher P., Ore. 76, 1941.

Babingtonia pestifera Syme in Sowerby, English Bot. ed. 3,9: 83, 1869 - published in synonomy.

Elodea canadensis var. latifolia Aschers. & Graebn. and forma latifolia Aschers. & Graebn., Synops. Metteleur. Fl. 1: 403, 1897.

E. canadensis var. Planchonii (Casp.) Farw., Am. Midl. Nat. 10: 302, 1927.
E. iowensis wylie (as Iowensis), Proc. Iowa Acad. Sci. 17: 82, 1910 - nomen provisorium.
E. latifolia Casp: Monatsber. Kgl. Preuss. Akad. Wissensch. 1857: 46, 1857 - published as a doubtful species, based upon sterile material.
E. oblongifolia Michx. ex Casp., Pringsheim's Jahrb. 1: 462, 1858 - published in synonomy.
E. Planchonii Casp., Monatsber. Kgl. Preuss, Akad. Wissensch. 1857: 47, 1857.

Philotria angustifolia Britton ex Rydb., Fl. colo. 15, 1906.

P. canadensis (Rich. in Michx.) Britton, Science II, 2:5, 1895.
P. iowensis Wylie, Proc. Iowa Acad. Sci.: 17: 82, 1910 - provisional name
P. Planchonii (Casp.) Rydb., Bull. Torrey Bot. Club 35: 462-463, 1908.

Serpicula canadensis (Rich. in Michx.) Eaton, Man. ed. 5, 390, 1839. In large part and as to basionym.

Udora canadensis (Rich. in Michx.) Nutt., Gen. N. Am. Pl. 2: 242.
U. occidentalis Koch, Sny. Fl. Germ. & Helvet. 669, 1837.

DESCRIPTION: Submerged aquatic, rooting in the mud; plants dioecious staminate plants rarely observed, their stems 2-10 dm. long; lower leaves opposite, small, lance-ovate; median and upper leaves in whorls of 3 and 7-17 mm. long, 1-4 mm. broad, linear, lance-linear, or lance-oblong, bright green, thin, firm or subflaccid, finely serrulate; staminate spathes borne in the upper axils, 15 mm. long, the lower half narrowed into a peduncle-like base the upper half 6-8 mm. long, 4 mm. wide, inflated, ellipsoid or ovoid, the apex resembling a gaping mouth, due to the two acute, salient lobes, staminate flowers peduncled by the slender, thread-like, elongating base of the hypanthium which is 3-20 cm. long; sepals 3.5-5 mm. long, 2-2.5 mm. wide, elliptic, dark striate; petals 5 mm. long 0.3-0.7 mm. wide, white, very delicate, the basal half a linear claw, the apical half linear lanceolate; stamens 9, the 3 inner raised on a common, fused stalk; anthers 2-3.5 mm. long, 1 mm. wide, oblong-ellipsoid; pistillate plants with stems slender, dichotomously branched; lower leaves opposite, ovate, much smaller than the upper ones; median and upper leaves in whorls of 3, and 6-13 mm. long, 1-5 mm. broad, oblong, ovate-oblong or lance-ovate, firm, dark green, serrualte, crowded and strongly imbricate at tip of the stem; pistillate spathe 10-20 mm. long, borne in upper axils, cylindric, the apex bidentate and with two broad teeth; pistillate flower exserted from the spathe by the thread-like, elongating base of the hypanthium which is 2-28 cm. long; sepals 2-2.2 mm. long, 1.1 mm. broad, oblong elliptic, dark striate; petals 2.6 mm. long 1.3 mm. wide, broadly elliptic-spatulate, white, delicate; staminodia three, 0.7 mm. long, acicular; style equaling the hypanthium; stigmas three, 4 mm. long, broad, 2-cleft at apex; ovary 3 mm. long, lance-ovoid; ovules 3-4, erect; capsules 6 mm. long, e.2 mm. in diameter, ovoid; seeds 4.5 mm. long, narrowly cylindric, glabrous (St. John, 1965).

DIAGNOSIS:

Flowers: Pistillate flowers hypogynous, axillary and perfect. Style - 2 lobed, stamens 3-9. Female flowers with long perianth tube. Flowers water pollinated. Absence of tubers and turions. Vegetative structure: Lower leaves alternate, opposite or whorls of three. Middle and upper leaves opposite or whorls of 3-7. Leaves 4-5 cm. long and 1.2-4.0 mm. wide. Leaves may or may not be copiously toothed.

Chromosomes: 2x = 24

Range: This species occurs in N.A. from Quebec southward to Virginia and Alabama, westward to Saskatchewan and Oklahoma. Plants also occurring sparsely west to Washington and northern California. This

genus is not known to inhabit Florida water. Also found in Mexico. In S.A. from Colombia south to Peru at high and low altitudes. Eastward from Colombia to French Guinea and south to Argentina.

HABITAT: This species exists in ponds, streams, lakes and canals especially in slowly moving water. This species occurs especially in calcareous bodies of water. Plants grow in mud, sandy muck and clay substrate.

EGERIA

Egeria densa: Planchon, Ann. Sci. Nat., Bot. III, 11: 80, 1849.

Type: Holotype - "in ditione Platensi, prope Bonarian; Tweedie in herb. Hook." (=Buenos Aires, Argentina)

SYNONYMS:

Anacharis densa (Planch.) Victorin, Contrib. Lab. Bot., Univ. Montreal 18: 41, 1931.

Elodea canadensis var. gigantea Hort., Bailey, Stand. Cyclop. Hort. 2: 1, 111, 1914.
Elodea densa (Planch.) Casp., Monatsber. Kgl. Preuss. Akad. Wissensch. 1857: 49, 1857.
E. densa var. longifolia Hort. ex. Bonstedt in Parey's Blumengaetnerei 1: 110, 1930.
E. gigantea Santos, Bot. Gay. 75: 44, 50, 1923.

Philotria densa (Planch.) Small, Man. S. E. Fl. 28, 1, 503, 1933.

DESCRIPTION: Submersed aquatic; stem stout, 2-3 mm. in diameter; lower leaves in whorls of 3, and 3-7 mm. long, 1.5-2 mm. wide, ovate or lance-ovate; middle and upper leaves in whorls of 4 or 5, and 12-40 mm. long, 1.7-5 mm. broad, oblong to broadly linear, acute, serrulate; nodes close together, but the leaves mostly divergent; flowers dioecious, staminate spathes borne in the upper axilis, 11-12 (-14) mm. long, 3.5 mm. broad elliptical, cleft down one side, the apex bifid with 2 short blunt teeth; staminate flowers 2-4 from each spathe, long stipitate, raised above the water by the slender, thread-like, elongating hypanthium which is 3-6 cm. long; sepals 3-4 mm. long, elliptic-oblong, boat-shaped, dark striate; petals 9.5-11 mm. long, 3.5-9 mm. wide, obovate to suborbicular, subacute at tip, cuneate at base, white, erect; stamens 9 (-10), distinct 2-2.5 mm. long, the central ones not connate; filaments heavy, clavate, flattened, glandular papillose above; anthers

0.5-1.2 mm. long, unequal; nectary central, green, 3-lobed, secreting abundant nectar; pistillate plants unknown to the writer, not represented in any of the numerous herbaria examined, including that of L. Hauman-Merck in Brussels, but described in detail by him while in Buenos Aires, and the following description is drawn from a translation of his article in Rec. Inst. L. Errera 9 (1913=1912) 34-35; pistillate spathe 10-11 mm. long, entire or slightly cleft along the median line, 1-flowered, calyx like the above, corolla smaller, the petals 8 mm. long, 8 mm. wide, less regularly plaited; the 3 staminodia 2.5 mm. long, filiform, sharp, yellow, covered with papillae, alternating with the branches of the style; style white, trifid to the base, with filiform lobes 3 mm. long, deeply 2-3 cleft and covered with papillae for their whole length; at the base of each lobe towards the outside, a little green nectary; ovary surmounted by a thread-like stalk 2-6 cm. long, 1 mm. in diameter; ovary cavity small, 1-celled, producing a swelling at the base of the neck; ovules 3-6, erect; fruit 7-8 mm. long, 3 mm. in diameter, obscurely trigonous, attenuate at the summit and surmounted by a vestige of the neck of the ovary; pericarp membranous, transparent; seeds 7-8 mm. long, (rare) sessile, fusiform, completely filling the locule owing to their swollen, mucilaginous seed coat and papillae, surmounted by a slender 2 mm. filament (St. John, 1961).

DIAGNOSIS:

Reproductive structures: Flowers raised above water. Two to four flowers arise from single spathe. Pistillate sex rare. Yellowish-white petals 1 mm. long. Style 3-lobed. Stamens 9-10. Flowers insect pollinated. Plants dioecious. Absence of tubers and turions.

Vegetative structures: Lower leaves opposite or whorls 3-7. Upper leaves whorls 4-8. Leaves 1-4 cm. long and 5 mm. wide. Leaves finely toothed.

Chromosomes: 2x =48

RANGE: Native land: Southern Brazil, Paraguay, Uruguay and Northern Argentina. Transplanted areas: United States - Mass., New York, New Jersey, Pennsylvania, Maryland, Virginia, N. Car., S. Car., Ga., Florida, Ken., Ala., La., Texas, Neb., Ark., Mo., Ariz., Oregon and California, Germany, France, Italy, Japan, New Zealand.

HABITAT: *Egeria densa* prefer moderately fast to fast flowing streams and rivers but it will grow in slow moving water or even still water. The plant roots in mud, sand, muddy sand or sandy mud.

HYDRILLA

Hydrilla verticillata Presl. Bot. Bemesck, 112, Ind. 1833-34.
Serpicula verticillata - first described by Linnaeus fillius (1781), put under family Haloragaceae.
Hydrilla ovalifolia - Richarel (1811) placed species in Hydrochoritaceae giving it the new specific epithet and added a description for the species.
Hydrilla verticillata - Presl. restored original specific epithet (1833-34). Royle (1839) gave short description. Caspary (1857) gave more detail.

TYPE: Holotype - Serpicula verticillata

SYNONYMS:

Hydrilla ovalifolia Rich. Man. Inst. Par., xii, Fr. ii, 76, t.z. 1811.

H. Roxburghii Steud. Nom. Bot. Pt. I, A-K, ed. II, 1, 780, 1840.
H. najadifolia Zoll and Moritzii. Syst. Verz. Zoll., 91, 1845-46.
H. angustifolia Hassk. Pl. Jau. Rar., 117, 1848.
H. muscoides planch. Ann. Sc. Nat., Ser. III, xi, 79, 1849.
H. Wightii Planch. Ann. Sc. Nat., Ser. 3, xi, 79, 1849.
H. angustifolia Bl. Mus. Bot. Lugd., Bot. i, 82, 1849-51.
H. dentata Casp. Bot. Zeit., 56, 1854.
H. verticillata Casp. in Monatsber. Akad. Berl. 1857.
H. japonica Miq. Ann. Mus. Bot. Lugd., Bat. iii, 271, 1867.
H. alternifolia Miq. Illus. Fl. Ind. Arch., 52, 1871.

Elodea canadensis var. crispa Hort. ex. whrh. Gartenstauden 1: 10, 1929.
E. verticillata (l.F.) F. Muell, Key Syst. Vict. Pl. 1: 423, 1888.

Udora lithuanica Andrz. ex Bess., Flora, Beibl. 1832. (2): 13, 1832.

U. verticillata Spreng. in Linnaeus' Syst. Veg., ed 16 by Spreng., 1: 170-171, 1825 basionym - H. verticillata
U. occidentalis Koch, Syn. ed. 1. 669 (1837).
U. pomeranica Reichb. Icones f. 104 (1845).

DESCRIPTION: A submerged leafy dioecious herb, leaves short, 3-4 nately whorled, or the lower opposite. Male flowers solitary, stoutly pedicelle, in a subglobose sessile muricate spathe; sepals 3, ovate or obovate, green; petals 3, oblong or cuneiform; stamens 3, anthers large, reniform, opening

elastically; pistillode small. Female flowers 1-2, sessile in a tubular 2-toothed spathe; perianth of the male, but leaflets narrower; ovary produced beyond the spathe in a filiform beak, 1-celled; styles 2-3, linear, undivided; stigmas 3, fimbriate; ovules anatropous. Fruit subulate, smooth or muricate; seeds 2-3 oblong, testa shortly produced at each end. Plant forming large masses. Leaves 1/4 - 1/3 in., 4-8, in a whorl, with a short sheathing one at the base of each branch and a short pair above this linear or oblong-linear, serrulate or entire. Flowers 1/6 - 1/4 in. long; perianth segments very variable. Fruit smooth or sometimes muricate, or (in Ceylon) squarrose with filaments above the middle. The male flowers escape from the sheath when mature, and float to the top of the water (Bennett, 1914).

DIAGNOSIS:

Reproductive Structures:

Pistillate flowers are epigynous. Entire style and 3 stamens. Flowers never perfect. Presence of tubers and turions.

Vegetative Structures:

Lower leaves opposite or whorls of 3-6 leaves, upper leaves in whorls of 4-8, leaves always copiously toothed.

RANGE: Found in Russia, Australia, New Zealand, Central Africa, East Asia, India, Europe and United States (Florida, S. Georgia and Alabama.)

According to these descriptions there are a few floral differences between the genera. E. densa has 2-4 large, yellow, staminate flowers on each spathe. H. verticillata and E. canadensis have one, solitary, smaller white flower on each spathe. In most other respects, (number of sepals, petals, stamens, etc.) the flowers of these plants are similar. E. densa flowers can be either insect or water pollinated whereas the other two genera are strictly water pollinated. All three genera reproduce mainly by fragmentation.

Vegetative morphology can be quite misleading. Most vegetative characteristics appear to be either environmentally controlled or too variable to be reliable. Internodal space and size of leaves are determined by several factors, mainly; rate of growth, depth of water, and height of plant. When a plant goes through a period of rapid growth the resulting internodal spaces are large until the plant reaches the surface of the water. At the surface the spaces become smaller. If the water level was to rise, the plant would

again enter a period of growth and the internodal spaces formed would again be large. This kind of growth would leave an area of close internodal spaces due to the previous water level. So the internodal spaces as well as the size of the leaves vary greatly on growing plants.

The number of leaves on a whorl also varies, especially on H. verticillata. Not only does it vary on different plants but the number varies at different points on the same plant without any set pattern. We have observed whorls of 2, 4, 5, 6 and 9 leaves on the same H. verticillata plant. In E. densa the number of leaves is not as variable, as we have observed whorls of 3, 4, 5, 6 and 8 leaves with most whorls of 4 and 5 leaves.

Serrations on the leaves have been used by many taxonomic keys to distinguish between the genera. H. verticillata has large, copiously toothed leaves, E. canadensis leaves are lesser or equally toothed and E. densa has smaller and more numerous serrations than the other two. The number appears to be a function of the length of the leaf with E. densa leaves larger on the average than H. verticillata leaves. Upon examination, we found that most of the teeth or spines were missing from the E. densa leaves which may explain why these leaves have been described as "finely toothed".

Scatter diagrams made from measurements of vegetative parts of the plants proved inconclusive. Measurements of plants that were illustrated and described in the literature produced some clearly defined diagrams, but measurements of plants obtained from the field produced diagrams with over-lapping of the genera.

Citations of possible interbreedings are inadequate, vague and in need of further research. More work also has to be done in areas of chemical and chromosomal taxonomy. One other area which did not appear in the literature was the presence or absence of tubers or turions. At the present time, H. verticillata is the only species known to have these structures. Lack of investigation may be the explanation for the lack of information on the other two genera.

The range of these plants does not seem to be restricted. Areas not presently inhabited by these plants could and possibly will be infested in the future. H. verticillata was previously believed only to inhabit South Florida, but since has been found in all parts of Florida, parts of Georgia, and Alabama and even in Iowa. All three plants grow in most types of soil and in most water conditions. These non-restrictive habitats and ranges cannot, at the present time, be used as taxonomic characteristics.

After total analysis, we see these three genera having most characteristics in common with only a few differing characteristics of slight variation. The basis of present classifications is flower structure with some use of vegetative characteristics. These genera and possibly the whole family of Hydrocharitaceae should be reorganized.

SECTION VI

Hydrilla verticillata

Hydrilla verticillata (Casp.) is a submersed caulescent, vascular hydrophyte, that is successfully colonizing the fresh waters of Florida. (Steward, 1969) Its presence in Florida was first noted in 1960, when it was mistakenly identified as elodea, the common fish aquarium grass (Elodea canadensis Michx.). Since that time it has infested over 45,000 acres throughout the state and has invaded southern Georgia and Alabama (Steward, 1969). A specimen of hydrilla was collected from Big Lake Conway in Orlando in 1962 and it appeared at Crystal River in 1964 where it has become a serious pest. The weed then spread to Lake Seminole near Chattahoochee in 1965 and in 1968 was reported in West Palm Beach (Ward, 1968). Since this time it has infested the canal systems of Broward and Dade counties to the point of impeding the water flow up to 90% in some. (Blackburn, White, and Weldon, 1968).

A number of chemicals have been field tested and found to be effective in the temporary control of this aquatic weed. (Blackburn and Weldon, 1966; Dean, 1968; Holm, Weldon and Blackburn, 1969). Attempts to obtain lasting control, however, have been unsuccessful. The resistance of this plant to control procedures is due in part to its ability to propagate vegetatively according to Steward. Intermittently along the length of the prostrate stem or stolon, lateral buds at nodes will germinate, grow and eventually give rise to additional plants. In addition to stolon growth along the surface, the growth of some are subterranean. After a short period of growth, the apex of the subterranean stolon undergoes a morphological change. The apical meristem of the stolon ceases in growth activity and becomes enclosed and protected by bud scales. The cells behind the meristem become swollen and enlarged because of a buildup of starch granules thus aiding in the protection of the meristem. This resulting swollen and enlarged portion of the stolon has been termed a "turion". This turion, which is capable of germinating and producing a normal plant, is believed to be one of the primary causes of reinfestation. The turion is adequately protected from herbicides present in the water by the layer of hydrosoil above it and because the starch reserves in the turion are able to support growth for 85 days or longer in the complete absence of light, any herbicide in the water has dissipated long before the turions germinate into new plants.

Along with chemical treatments for hydrilla control, mechanical harvesting has been used extensively, but for the most part the initial cost outlay is prohibitive for small cities and townships and the results are at best a masquerade. Physical control of hydrilla has been tested in some areas using concentrated sulphuric acid in order to lower the pH of the water and although this effectively killed the weeds, other side effects were noticed which caused termination of the project (Hyde, 1969).

One of the most promising tools in the realm of aquatic weed control is the use of biological agents. Our own university saw the initiation of biological control research when Sguros (1965) investigated the use of seacows (Trichechus manatus latirostus) as weed harvesters. In addition the possibility of using weed-eating snails (Marisa cornuarietis and Pomacea australis), grass eating carp (Ctenopharyngodon idella), and weed-eating crayfish (Orconectes causeyi) to control hydrilla and other noxious weeds have been investigated by numerous workers (Cross, 1969; Dean, 1969; Kaniuka, 1968; Seaman and Porterfield, 1964).

MATERIALS AND METHODS
(*Hydrilla verticillata*)

GROWTH OF ENTIRE PLANT

Of primary importance in the study of any organism is the cultivation of that organism in a controlled environment. This is especially true in attempting to isolate pathogens from the organism. In order to satisfy Koch's postulates, healthy, controlled hosts must be available for reinoculation. One must understand or at least recognize the influence of climate on the physiological profile of aquatic weeds, and on the subsequent performance characteristic of a particular microbial pathogen.

In studies of the microbiological control of aquatic weeds conducted at Florida Atlantic University, cultivation of healthy *Hydrilla verticillata* was investigated. In addition, studies on the feasibility of surface sterilization were initiated to enhance isolation of suspected pathogens. Once these procedures were established they were applied to the leaf whorl experiments.

Initially plants were grown in outdoor open wading pools. The pools were filled with water from a neighboring canal and sand from the canal bank was added to a depth of three to four inches. Normal appearing plants were planted in the pools and periodically observed.

Cultivation of *Hydrilla* in the laboratory was then attempted. Ideally the laboratory growth conditions should parallel those occurring in nature, however, certain limitations of equipment may require modification of these in situation environmental conditions. In addition, it is sometimes necessary to alter environmental conditions to maintain or induce a particular physical state of growth. Experiments were designed to develop a practical system for sustained growth of *Hydrilla verticillata* in Percival environmental chambers. These controlled environmental chambers allow one to evaluate and repeat at will the single or combined influence of various climatic factors including light, temperature, and humidity on growth. These experiments were not designed to establish optimal growth conditions or exact nutrient requirements. The main purpose of these studies was to develop a controlled and standardized system which could be applied to a variety of experimental situations.

An environmental chamber was programmed for a twelve hour day/night cycle. The night/day temperature range was 21 to 27 C with high humidity and constant positive air circulation. The light intensity was 2000 foot-candles provided by both incandescent and fluorescent lamps. Two ten gallon aquaria

containing a sand bottom and filled with canal water were placed in the chamber. Hydrilla and Alternanthera philoxeroides were planted in the aquaria. The canal water contained various invertebrates and small aquatic plants.

However, the need for isolated normal Hydrilla was not satisfied by this system. Research was initiated to establish conditions for sustained vegetative growth of Hydrilla in the absence of other forms. Hydrilla was planted into ten gallon aquaria in large populations and in specimen jars as individual plants of two stolons. Four nutrient levels were studied: 1) distilled water; 2) 0.01% Hoagland's solution; 3) 1.0% Hoagland's solution; 4) 50% Hoagland's solution. Three temperature ranges 16-21 C, 21-27 C, and 27-32 C were tried all with a high humidity and positive air circulation. Light intensities of 2200, 1800, 800, and 40 foot-candles were investigated.

SURFACE STERILIZATION

The next step was to develop techniques for surface sterilization of Hydrilla to aid in re-isolation of suspected pathogens. Initially a surface sterilization technique was used which had been developed by our laboratory for isolation of internal organisms. This consisted of a 15-20 second rinse in Tween 80 and water, a 5 second dip in 5% Mercuric chloride, a 15 second rinse in 0.5 M Sodium thiosulfate, and three sterile water rinses.

The next substance tried was Alkyl dimethyl dichlorobenzyl ammonium chloride which is a quaternary germicide for extremely hard waters. Quaternary germicides have the advantage of effectiveness at very low concentrations and availability of chemical inactivators. Plants were rinsed in sterile distilled water, dipped activator solution of lecithin and finally rinsed in three sterile distilled water baths. The stolons were planted in jars of sterile 0.01% Hoagland's solution and a small portion of the stolon removed and placed in letheen bacteriological recovery media for quaternary germicides.

LEAF WHORL CULTURE

Detached leaf cultures, the maintenance of excised leaves or leaf parts in fluid medium, have been used in the study of obligate parasites in several plant diseases. (Yarwood, 1946). The life of detached leaves is prolonged where callus tissue is formed (Turel, 1957) or where rooting occurs (Chibnall, 1954). With wheat, in which neither of these occurs Person (1957) reported that detached leaves can be maintained for relatively long periods when floated on benzimidazole solution. Samborski (1958) showed that with appropriate concentration of this chemical, typical rust reactions could be obtained.

Leaves floated on benzimidazole showed no appreciable changes in levels of protein, amino acid, or alcohol soluble carbohydrate as compared with those floating on water. It was obvious that the method would be useful in studies of host-parasite relations. Initial studies by our laboratory demonstrated that individual detached leaves of Hydrilla became debilitated within 24 hours in a variety of culture solutions. The maintenance of whole leaf whorls in liquid culture was then attempted.

Freshly collected plants of Hydrilla verticillata were rinsed in distilled water and examined for signs of disease. Whorls appearing to be in the same physiological state were cut from the stolon and placed in 100 mm. glass petri dishes containing 30 ml. of test fluid. After being immersed in the fluid, excess lengths of stolon were trimmed below the surface of the fluid to within 1 ml. of the leaf blade base. The plates were placed in an environmental growth chamber programmed for a twelve hour day/night cycle. The night/day temperature range was 18-24 C with a light intensity of 40 foot-candles.

Five growth media were tested: 1) distilled water; 2) 50% Hoagland's solution; 3) 0.01% Hoagland's solution; 4) 50 ppm Benzimidazole; 5) 50 ppm Benzimidazole and 0.01% Hoagland's solution.

LAKE WALES STUDY

With procedures for cultivating entire and partial Hydrilla plants established and methods for ridding endogenous contaminants worked out, the efforts of our laboratory were directed toward the microbiological control of Hydrilla, i.e., the use of fungi, bacteria or viruses, which led us to an investigation of a freshwater lake in central Florida. This body of water, Lake Wales in Polk County, has long served an integral aesthetic and recreational role to the tourist-oriented city located around its bank and bearing its name. It has an area of approximately 300 acres and reaches depths of 30 feet in the western half.

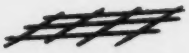
Hydrilla was first observed here in 1967 by the Florida Game and Fresh Water Fish Commission. At that time it was referred to and misidentified as the genus Elodea. They indicated it was an exotic species and suggested that it had probably been intorduced from a fish aquarium, most likely in the cove located on the eastern portion of the lake. In December of 1968, the Game and Fish Commission estimated that 18 acres (6%) were infested; primarily around the shoreline and recommended the application of copper sulphate at the rate of 100 pounds per acre. Following the Commission's advise the city applied the copper sulphate and noticed what they thought was a recession of the weed, but found out later that it was actually the normal winter die-back. It is now mutually agreed that the copper sulphate treatment had no drastic effect on the Hydrilla infestation.

FIG. 1. Graphic representation of Lake Wales depicting the area where the Hydrilla was suspected to originate, the areas of chemical treatments, and the areas where the plant and water samples were collected.

LEGEND

Area where Hydrilla was thought to originate _____

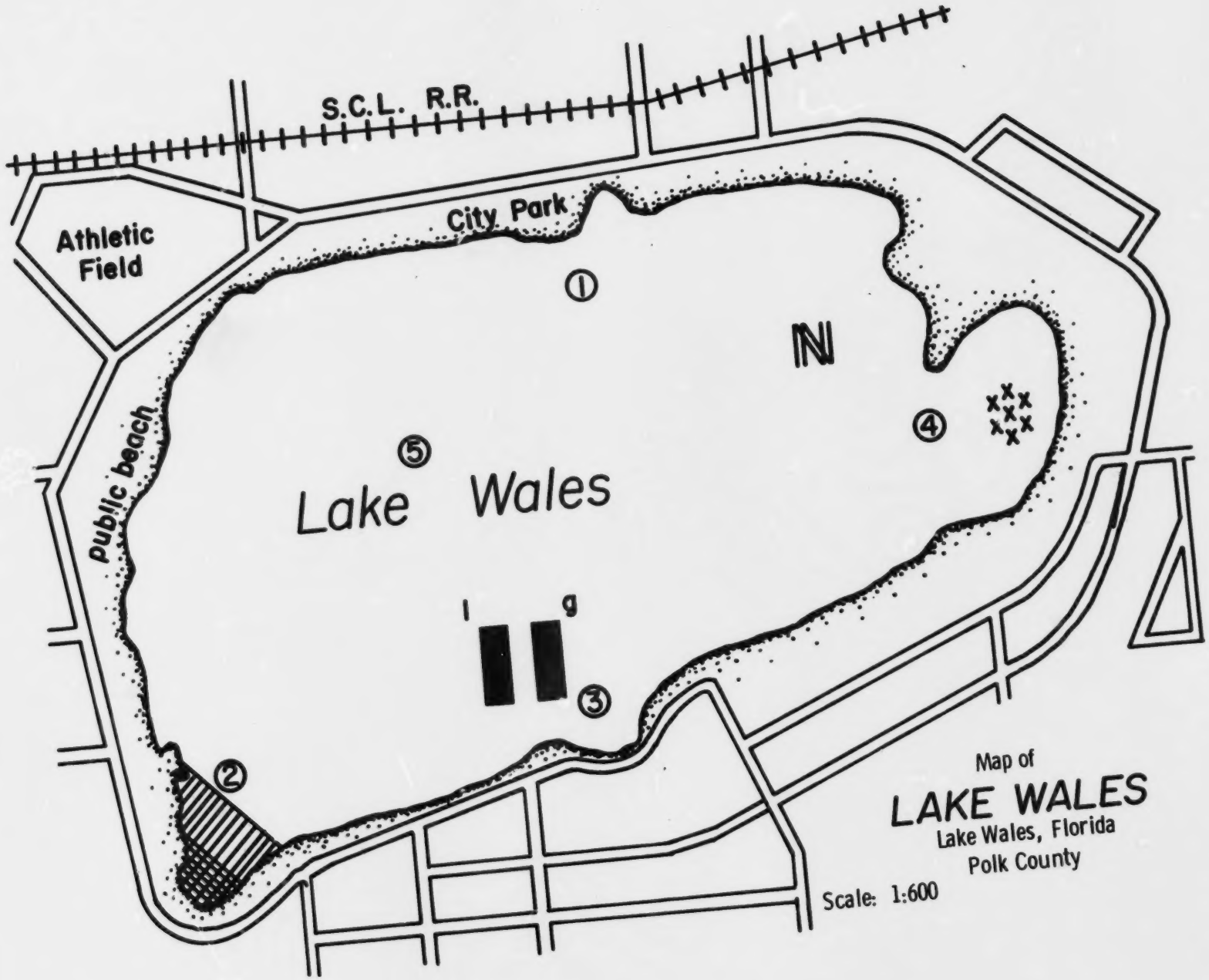
Area where the copper sulphate treatment was applied _____

Area where the copper sulphate-diquat treatment was applied _____ 

Area where the Hydrothal 191 treatment was applied _____

Areas where plant and water samples were collected:

Normal _____	1
Chlorotic _____	2
Chlorotic _____	3
Chlorotic _____	4
Normal _____	5



Map of
LAKE WALES
Lake Wales, Florida
Polk County

Scale: 1:600

This is somewhat surprising since copper sulphate, like salts of other metals appear to inactivate enzymes and precipitate proteins in plants (Brian, 1964). However, it has recently been shown (MacKenzie and Hall, 1967) that a combination of copper sulphate and diquat [6,7-dihydrodipyrido (1, 2-a: 2', 1'-c) pyrazinesium ion] resulted in a better control of aquatic plants, especially the submersed ones like Hydrilla than when the herbicides or metallic salts were applied individually. The cause of this apparent increase and selective phytotoxic effect of the combination compared to the phytotoxic effect of the individual herbicide is not known at present but it has been suggested (Sutton, Weldon, & Blackburn, 1970) that the combined products alter the permeability of the cell membrane thereby increasing the amount of copper sulphate that enters the cells.

By early summer of 1969, more than 180 acres (60%) of the shoreline out to a depth of 18 feet of water was infested and the city looked into the possibility of chemical treatment and/or mechanical harvesting. Several of the city's officials made trips to Winter Park, Florida to examine their mechanical harvester and at that time had a bid from a contractor of $18,000 to harvest the Hydrilla up to 100 feet from the shoreline. After much discussion and deliberation, the city decided that mechanical harvesting did not solve the problem; and was in effect spending good money after bad.

In July of 1969, the city of Lake Wales and Polk County jointly tried an application of the diquat and copper sulphate combination in a one acre test plot in the southwest corner of the lake (Fig. 2). The result was a substantial reduction in the Hydrilla with no apparent adverse effects. Unfortunately, the cure was only temporary because within six weeks the weed had regrown almost to its original density.

At the same time of this copper sulphate-diquat treatment, a chemical supplier put out two test plots of Hydrothal 191 which has as its active ingredient endothall acid (7-oxabicyclo [2, 2, 1] heptane -2, 3-dicarboxcyclic acid). The two test plots were 150 foot strips approximately 100 yards east of the cove and 20 yards offshore (Fig. 2.). One strip received the Hydrothal in granular form and the other in liquid form. The results were very similar to those obtained with diquat, i.e., a substantial but temporary Hydrilla kill; however, a small fish kill resulted apparently from the granular application. Even though several applications of the chemicals could conceivable control the weeds, the expense for treatment of the entire lake is prohibitive (approximately $30,000).

By the summer of 1970 it was estimated that better than 90% of the lake was infested with Hydrilla which resulted in the termination of all recreational activities on the lake and forced the city to close the lake to bathers.

In February 1971, the Southwest Florida Water Management District experimented with a bi-fluid system on a three acre test cove that overlapped the previous test area (Fig. 2). Their bi-fluid system contained an oil phase: water phase ratio of 6:1. Five gallons of xylene to one gallon DPN made up the oil phase while the water phase contained one gallon diquat: 20 pounds copper sulphate: 50 pounds Ammate X.[1]. They repeated their application in June 1971 and had good results; however, when we visited the lake four months later in October, the test cove was completely reinfested with Hydrilla. Not only was the Hydrilla back, but as Fig. 3 shows, an algae bloom (Cladophora spp.) was occurring covering the exposed Hydrilla tips and forming a dense mat which was allowing alligatorweed (Alternanthera philoxeroides) to invade the lake.

This then brings us to the reason we investigated Lake Wales. It was brought to our attention that the lake experienced a noticeable regression in the Hydrilla infestation about a year ago for no immediately apparent reason. Biologists from the Fish and Game Commission were asked to examine the lake but evidence for a responsible biological agent could not be found. Our initial observations indicated a general chlorosis or yellowing of the top portion of the plants in several large areas of the lake. Since normal growth in plants is under hormonal control by such physiological active compounds as indoleacetic acid, the gibberellins, etc. a departure from the normal levels of these compounds in host cells, such as may be experienced in a disease situation, could change the growth and development of the host. Because chlorosis is one such change and it is one of the most common and most noticeable symptoms of plant disease samples were gathered of the plants and surrounding water for investigation.

Sampling was performed at five different locations on the lake (Fig. 1). Numbers one and five were selected as our control or normal samples since there was no indication of excessive chlorosis in these areas. On the other hand, locations two, three and four showed extensive chlorotic plants. Plant samples were collected in plastic bags containing water taken from the same location while water samples were collected in sterile test tubes (16 mm. x 125 mm.). All samples were transported under refrigerated conditions via a styrofoam ice chest.

Isolation of the microbial flora from the plants was accomplished by first surface sterilizing them through a series of dips and rinses and then homogenizing in a sterile buffered saline solution. Serial dilutions to 10^{-6} were made into each of three growth media. Pour plates were made in duplicate, one set being incubated at 24 C and the other at 35 C.

RESULTS

(*Hydrilla verticillata*)

GROWTH OF ENTIRE PLANT

The plants grown in outdoor pools rapidly deteriorated due to the heating of the water by the strong direct sunlight. Wooden structures standing six feet tall and built of wooden slats were placed over the pools which provided approximately a 50% reduction in sunlight. These pools have served as a readily accessible source of *Hydrilla*.

In the environmental chamber studies a small balanced ecosystem was established. After several weeks a balance of organisms was observed in which the *Hydrilla*, the *Alternanthera*, and various smaller plants such as *Lemna minor* appeared to be prospering. The miniature ecosystem was complete with damsel flies and crayfish. The system was entering its eighth month of existence without apparent dominance of any one species.

It was noted that the *Hydrilla* planted in large populations in the aquaria were less influenced by changes in environment than the individual stolon pairs in the specimen jars. The outer layer of plants in the aquaria acted as an environmental shield and the larger volume of water minimized differences in conditions.

There was no apparent growth differences between the three temperature ranges tried. A temperature range of 18-24C was chosen due to the less evaporation occurring at this level. All temperatures studied appeared to be within the effective growth range of *Hydrilla*.

In nature, light is considered the most variable and crucial physical condition for aquatic weed growth - this held true in the environmental chamber studies. Stolon pairs exposed to the 2200 and 1800 foot-candle light intensities rapidly became achlorophic and debilitated. Those exposed to 800 foot-candles fared better initially but eventually were also debilitated. Specimens in 40 foot-candles prospered and demonstrated sustained growth. The low light requirement of *Hydrilla* is similar to the low light requirement of *Elodea* as reported by Blackburn (1961). He found that under the conditions of his experiment optimal light intensity using white fluorescent lamps was somewhere near ten foot-candles.

Stolons grown in the 50% Hoagland's solution soon became overgrown with algae, with increasing time the 1.0% Hoagland's also became overgrown with algae. The slow sustained growth which was desired occurred both in the 0.01% Hoagland's and the distilled water, under low light conditions.

SURFACE STERILIZATION

In previous experiments this technique had proven to be very effective when the surface sterilized plant did not need to remain viable. The treated stolons turned a brown color and appeared to be dead; however, after approximately one week about a third showed new growth. Considering the shock to the plant and the time for recovery this technique was discontinued. Surface sterilization using 5% Sodium hypochloride also proved too severe.

Sterilization using the alkyl dimethyl dichlorobenzyl ammonium chloride proved to be more successful. The stolons remained green and no interruption of growth was observed. The cultured sections demonstrated no growth in the recovery media. Culturing of the growing plants several weeks after sterilization showed some stolons to have surface bacteria present. This may be due to a number of reasons: 1) bacteriostatic action of the quat on certain organisms; 2) migration of internal bacteria to the surface; 3) contamination from the outside. Contamination by algae was completely thwarted by this treatment. Our laboratory now had a system for inoculation of suspected pathogens into surface sterilized pure cultures of Hydrilla verticillata which could be maintained for long periods of time.

LEAF WHORL CULTURE

The best results were obtained using the 50 ppm benzimidazole and 0.01% Hoagland's mixture. The vast majority of whorls kept a normal green color and displayed cyclosis when examined microscopically. After approximately one month, 90% of these whorls showed new growth in the form of new leaf sprouts. Whorls grown in 0.01% Hoagland's alone showed a breakdown in green color, but also sprouted in about one month. The whorls in 50 ppm benzimidazole alone retained good color, but fewer whorls sprouted. Whorls in distilled water showed a variety of green coloration and browning; however, some did sprout. All whorls in the 50% Hoagland's solution became contaminated with algae and died. Leaf whorl cultures grown at light intensity higher than 40 foot-candles rapidly became achlorophic and debilitated. The low light requirement of the leaf whorl cultures of Hydrilla is similar to the low light requirements of the entire plant (Blackburn, Lawrence & Davis, 1961). Leaf whorl cultures of Hydrilla are currently being inoculated with suspected pathogens in the search for a microbiological control for this aquatic weed. This system proved to have the advantage over whole plants of greater economy of space, plant material, and inoculum; ease of manipulation and observation; and control of environment and puriety of pathogen biotypes. It is hoped that this economical system for vegetative growth of Hydrilla will enable a more extensive examination of microbiological control.

LAKE WALES STUDIES

At this time, identification of each organism isolated is still incomplete, since we were interested mainly in the possible pathogenicity of any isolated organisms rather than a total and complete generic identification of each; however, we have tentatively placed most of the isolates into their respective orders and families. Location number one which was a healthy appearing, normal area with a luxuriant growth of Hydrilla yielded four different species of bacteria, three of which we have indicated as belonging to the family Enterobacteriaceae of the order Eubacteriales and the other to the family Pseudomonadaceae of the order Pseudomonales. Locations two and three, which were the achlorophic regions produced 7 and 8 species respectively, four of which in each area were also found in the first location. The remaining 3 species in location two and the 4 species in location three were classified as pseudomonads producing a variety of pigments ranging from snow white to deep orange. The fourth location also showed drastic signs of chlorosis and resulted in four species which are presumably the same as those isolated from the second normal area, location five. These organisms are gram negative non-pigmented rods and have not been classified as yet.

These organisms with possible pathogenic potentials were subsequently introduced onto new Hydrilla plants and monitored for a period of weeks. Symptomatic areas of chlorosis appeared after several days, in those plants grown under high light intensities; however, the same organisms were not capable of producing chlorosis in Hydrilla plants maintained in low light. In addition, we were unable to isolate the organisms back from the chlorotic plants leaving the last of Koch's postulates unsettled. It is now believed by our laboratory that the symptomatic areas of chlorosis resulted from, in part, to a peculiar physical phenomenon known as photooxidation and not a direct result of an invading organism; thus suggesting the bacteria isolated from Lake Wales are most probably commensal in nature or at best saphrophytic living on an already slightly debilitated host.

SECTION VII

Eichhornia crassipes

A floating aquatic is one which stays on the surface of the water and does not generally root itself or attach itself to the bottom or the bank, but is free to move about with the current or wind. The floating weed with which most of us are familiar is the water hyacinth, Eichhornia crassipes. From all accounts we are relatively sure it is a native of South America, either of Peru or Brazil. It has spread from there to nearly all of the South American and Central American countries. The weed now occurs in nearly all the countries in the world whose climate is favorable to its development such as Australia, China, India, Indo-China, Japan, Siam and South Africa.

The accounts differ somewhat regarding its appearance in the United States. There is some evidence that it was cultivated as a greenhouse exotic shortly after the War Between the States. However, the first authentic account details its introduction at the Cotton Centennial Exposition at New Orleans in 1884. From New Orleans some of the plants were cultivated in ponds and gardens as admirable aquatic specimens. It is supposed that they were cast out or probably dumped into some nearby stream. The plant made its appearance in Florida in 1890 and has since become an important aquatic plant pest. Shortly after the turn of the century it had been reported from all the southeastern coastal states as far north as Virginia and was all the way west to California.

The relatively rapid rate of colonization by water hyacinth has been reported to be second only to a species of bamboo which is capable of 1-3 feet of growth per day. Most experiments placed Eichhornia in the range of doubling its size and mass every 11-15 days. Taking an average rate of doubling of once every 2 weeks and a growing season of 8 months, then 10 plants, given plenty of room and good growth conditions would produce 655,360 plants (Penfound and Earle, 1948).

The water hyacinth was officially recognized as a serious aquatic pest on June 4, 1897, when Congress passed an act authorizing the Secretary of War to investigate the extent of obstruction to navigation in the waters of Louisiana and Florida. Since this time the Army Corps of Engineers and the Florida Flood Control have attempted to keep the canals cleared.

MATERIALS AND METHODS

(Eichhornia crassipes)

GROWTH OF ENTIRE PLANT

Laboratory growth of Eichhornia crassipes was conducted both in an outdoor environment and in environmental growth chambers to provide a conveniently accessible source of healthy aqautic weeds to be used in pathogenecity experiments.

The preliminary attempt to grow plants in a controlled outdoor environment involved individual plants in buckets, fertilized with small amounts of commercial fertilizers.

Plants were also grown in two foot deep, six foot in diameter wading pools and in environmentally controlled growth chambers.

SURFACE STERILIZATION

In order to aid the recovery of possible pathogens for E. crassipes, methods were investigated for the preliminary surface sterilization of leaves. Apparently healthy, luxuriant E. crassipes plants were selected from a naturally occurring infestation between 1100 and 1130 hours during mid-June and transported to the lab. Eighty leaves were selected at random from the plants for each of the six treatments, the petiole was severed near the corm, and the blade rinsed initially in aqueous Tween 80 solution. Each group of eighty leaves was treated by a five second dip in either 1.25% v/v NaHClO, 5.25% v/v NaHClO (commercial Clorox), 1.0% w/v $HgCl_2$, 5.0% w/v $HgCl_2$, or absolute ethanol. All except those leaves dipped in ethanol received three consecutive rinses in distilled water of 10 sec. each, the ethanol-dipped leaves were ignited from an alcohol burner. A control group was subjected only to the Tween 80 and three distilled water rinses.

Following treatment each leaf was plated on one of the following microbiological culture media: 1. V-8 Juice agar; 2. Potato Dextrose agar (Difco); 3. Tryptic Soy agar (Difco); 4. Nutrient agar (Difco). The media were contained in 60 x 50 mm. sterile, plastic, disposable petri dishes (Falcon). The leaves were shaken free of excess water, the upper surface of the blade was applied to the medium surface, then gently pressed against the surface with the base of a 50 ml Griffen low form beaker to insure uniform contact. The plates were recovered and incubated at 27 C for the required length of time. Twenty leaves were utilized for each medium. The resultant colonies were counted within a 1 cm. square in the center of the plate on a Quebec colony counter. (Fig. 4). In addition, fungal colonies recovered were recorded, although no attempt of

identification was made (Table 1).

DETACHED LEAF CULTURE

Throughout these investigations apparently healthy E. crassipes plants with leaves ranging in diameter from 7 to 10 cm. were used. The plants were collected during the late afternoon from naturally occurring infestations in drainage canals near the campus of Florida Atlantic University in Boca Raton, Florida. Initial excision of the leaf was effected by pinching off the petiole near the corm of the plant. Subsequent manipulations were performed by holding the leaf by the petiole which remained to avoid mechanical damage to the blade. A second excision was performed at the time the leaves were placed into the respective test solution. This was accomplished by submersing the junction of the petiole and blade below the surface of the medium, where applicable, and severing the petiole using alcohol flamed scissors. This submersion precaution avoids entrapment of air in the severed vascular tissues which would hinder the translocation of media through these vessels.

Autoclaved (15 psi, 15 min.) 100 X 15 mm petri dishes were employed for culture except where presterilized plastic dishes were used. All dishes were covered and test solutions were adjusted to approximately original volume at 48 hr. intervals with demineralized water to compensate for evaporation. The solutions were replaced weekly by decanting old media while adding fresh media to avoid exposing the excised portion of the blade to air. Test solutions were not autoclaved, instead sterile containers and sterile demineralized water was employed for preparation. Commercial chemicals of reagent grade were used unless otherwise noted. Demineralized water was prepared by passing distilled water through a Crystalab Deeminizer demineralizer.

The procedure followed four general steps:
1. Manipulative investigations; 2. Preliminary medium study; 3. Biochemical comparison of selected media and

1. Manipulative investigations.

To determine the optimal techniques for culturing detached leaves of E. crassipes, five leaves were prepared for each of four test procedures. All cultures for this investigation employed the same medium of 50 ppm benzimidazole (1, 3-benzodiazole). The medium was selected on the basis of preliminary studies which showed a delayed senescence of E. crassipes leaves as compared to distilled water. Leaves for the first procedure were cultured in glass petri dishes by floating the leaf on 13

to 18 ml. of medium. The second technique was identical to the first except plastic petri dishes were used in place of glass dishes. A similar technique was investigated in the third procedure except glass petri dishes containing 5 to 7 ml. of medium were tilted such that the petiolar excision was immersed in culture medium and the remaining blade was free of the fluid. An angle of inclination of approximately 30 degrees was used for each plate. The last technique investigated employed glass petri dishes containing a sterile 60 mm square filter paper pad (3-5 ml.) and a leaf was placed on the moistened filter paper.

The leaves, which were obtained from a natural environment, exhibited debris (sand, muck, twigs, insects, larvae, etc.) on their surfaces. Although this study was designed to investigate only culture media, not surface treatments, the removal of this debris was apparently necessary for clean uniform cultures. A comparison was, therefore, performed between no pretreatment, pretreatment in Tween 80 solution (3 drops per 2 liters of water), distilled water rinse, and dilute clorox solution (1:5 v/v dilution of commercial Clorox-5.25% sodium hypochlorite). The clorox rinse was followed by two consecutive rinses in distilled water. Pretreatments were performed by grasping the leaf by the petiole and submersing the blade along with gentle agitation, into 1 liter of the test solution for 10 sec.. The solutions were changed whenever an accumulation of debris was noticed.

2. Preliminary media study.

To determine the optimal media or medium from the list of possible agents, a series of five leaves each were prepared according to the procedure described above employing glass petri dishes, 13 to 18 ml. of medium, and Tween 80 - dilute clorox pretreatment. Eight media were selected for study; distilled water, tap water, Hoagland solution (Tuite, 1969), 2% w/v sucrose solution, benzimidazole 50 ppm solution in demineralized water, benzimidazole 50 ppm solution in 2% sucrose, kinetin (6-furfuryl aminopurine, Nutritional Biochemicals Corp.) 50 ppm solution in demineralized water, and kinetin solution 50 ppm solution in 2% sucrose. The leaves were maintained at room temperature (22-25 C) under a light intensity of 100 ft candles (measured with a Weston Sunlight Illumination Meter, model #756) provided by soft white fluorescent tubes at a photoperiod of 12 hr. light. The appearance of the leaves was noted at weekly intervals as a color value rating by which the color value of normal leaves was rated as "5" and leaves devoid of green as "1". Termination of the experiment was determined by failure to note significant change in the group after one week or upon noting a condition of "2" which was indicative of a late stage of senescence.

3. Biochemical comparison.

The results from the preliminary media study failed to establish one optimal culture medium, therefore, biochemical differentiation was sought between the two optimal culture media, kinetin and benzimidazole. Two concentrations of each medium were compared, 50 and 100 ppm, as well as a mixture of the two agents in a final concentration of 50 ppm each. The biochemical parameters selected for comparison were total carbohydrate, alcohol insoluble nitrogen, and the water soluble nitrogen of the ethanol soluble fraction.

A naturally occurring population of E. crassipes was selected to impart homogeneity in selection and preconditioning by the environment. One hundred seventy five plants were selected from Lake Worth Drainage District Canal L-21 at its intersection with Military Trail in Palm Beach County, Florida. The plants were transported a distance of 5 miles to the experimental site. For each test medium 23 leaves were prepared and 14 for the distilled water control according to the procedure employed for the "preliminary media study". Environmental parameters were also similar to those employed earlier except the initial light source consisted of two soft white and two Gro Lux flourescent tubes at an intensity of 100 ft. candles, measured at the surface of the plates. After one week the Gro Lux tubes were extinguished and the light adjusted to retain the original intensity. Temperature was monitored with a Baccharach tempscribe 7-day recording thermometer. Five leaves were pretreated as the rest then sampled for biochemical analysis as a base-line value. Three leaves from each test medium were selected at 7-day intervals and sacrificed for biochemical analysis.

A sample as uniform in area as possible was obtained from each leaf for analysis by employing a #7 cork borer to remove 15 discs from each leaf. Each group of 15 discs were placed in a 26 x 60 mm disposable, cellulose, single thickness extraction thimble (Whatman) immediately upon sampling and frozen and thawed once to disrupt the tissues. The thimbles were placed in a soxhlet extraction apparatus and refluxed 2.5 hr. in 80% ethanol. The alcohol fraction was evaporated to dryness in wide mouth jars on a warming tray @ 50 C in an air stream. The resultant residue was dissolved in 10.0 ml of demineralized water, clarified by low-speed centrifugation and the supernatant estimated for carbohydrate and nitrogen. The tissue remaining in the thimble was dried at room temperature and employed "in toto" for the estimation of insoluble nitrogen levels.

A. Nitrogen content.

Nitrogen was estimated by a modification of the nesslerization micro-Kjeldahl procedure of Koch & McMeekin (White & Frankel, 1965). The sample, either all the dried, extracted

tissue, or 2.0 ml of the aqueous extract, was hydrolyzed. The sample was contained in 50 ml thick-walled Pyrex tubes to which 1.0 ml of 50% sulfuric acid was added for the tissue or 3.0 ml of 50% sulfric acid for the aqueous extract. The tubes were heated in a Bunsen flame until dense white fumes filled the tube, approximately one minute, to effect hydrolysis. The tubes were allowed to cool, 5 drips of 30% hydrogen peroxide was added, then the cleared solution was reheated three minutes to void excess hydrogen peroxide. The resultant solution was diluted with demineralized water to a measured volume sufficient for the range of the test. To 3.5 ml of the diluted solution was added 1.5 ml of Nessler's reagent (Sigma Ammonia Color Concentrate, Sigma Chemical Co.) and the optical density measured at 480 nm on a Bausch & Lomb Spectronic 20 spectrophotometer employing B & L 1/2" cuvettes. The values were compared with a standard curve prepared with ammonium sulfate. Known concentrations of ammonium sulfate and Monitrol I normal range serum control (Dade Reagents), a protein standard, were incorporated with each run as a control for the test.

B. Carbohydrate determination.

One ml of the aqueous extract was cleared of protein by precipitation with zinc sulfate according to the method of Somogyi (White & Frankel, 1965) in which the aqueous extract was diluted with 5.0 ml of distilled water and mixed with 2.0 ml of 5% zinc sulfate followed by 2.0 ml of 0.3 N barium hydroxide. The suspension was centrifuged at low speed and the supernatant used for carbohydrate determination by the anthrone technique of Yemm and Willis (1954). The anthrone reagent was produced by Marstan Chemical Laboratory, Delray Beach, Florida. The test solutions were heated for three minutes in a boiling water bath and the absorbancy measured at 630 nm on a Bausch & Lomb Spectronic 20 spectrophotometer employing B & L 1/2" test tubes. The data were compared with a standard curve of glucose. A known glucose solution was included in each run as a control for the test.

TISSUE CULTURE OF PLANT PARTS

The first medium tested was suggested by Dr. J. X. Hartmann (Department of Biological Sciences, Florida Atlantic University) for its broad success in plant tissue culture. Designated "B", its constituents are listed in Table 1. To this medium were added antibiotics and a fungiscide to inhibit anticipated contamination. Their proportions are described in Table 2. After autoclaving, the heat labile components were added and medium poured on plastic disposable Petri plates. All organic constituents except the sugars and 2,4-D were kept frozen (-10C) until needed.

TABLE I

CHEMICAL COMPOSITION OF MEDIUM "B" USED FOR TISSUE CULTURE OF *E. CRASSIPES*

Mineral Elements	mg/l	Organic Constituents	mg/l
KCl	26	Dextrose	800
$CaCl_2.2H_2O$	264	Sucrose	1800
KNO_3	1172	Glycine	2.4
$Ca(NO_3)_2.4H_2O$	120	Nicotinic Acid	0.5
NH_4NO_3	990	Thiamine HCl	0.1
$MgSO_4.7H_2O$	510	Pyridoxine HCl	0.3
Na_2SO_4	80	Myo-inositol	60
$NaH_2PO_4.H_2O$	8	2,4-D	0.1-0.25
KH_2PO_4	102	Indole acetic acid	0.6-0.8
$FeSO_4.7H_2O$	17	Napthalene acetic acid	0.04
$MnSO_4.4H_2O$	16	Calcium pantothenate	1.0
$ZnSO_4.7H_2O$	8	Kinetin	0.03-0.4
H_3BO_3	4	Coconut water	50 ml
KI	0.8	Na_2EDTA	23
		Agar	10,000

TABLE 2

ANTIBIOTIC AND ANTIFUNGAL AGENTS USED IN TISSUE CULTURE MEDIA FOR *E. CRASSIPES*

Penicillin

Buffered Potassium Penicillin G for injection

U.S.P. 5,000,000

From: Ely Lily and Co., Indianapolis, Ind., 46206

Stock Solution

Dissolve contents of ampoule in 50 ml sterile water for injection. Keep stock solution at -20°C.
Concentration - 100,000 units/ml

Working Solution

Use 0.25 ml of stock per 100 ml tissue culture medium.
Final concentration - 250 units/ml.

TABLE 2 (Con't.)

Streptomycin

Streptomycin Sulfate U.S.P. 5 gr.

From: Ely Lily and Co.

Stock Solution

Dissolve contents of ampoule in 17 ml. sterile water for injection. Concentration-250 mg./ml. Store at -20 C.

Working Solution

Use .1 ml. of stock solution per 100 ml. tissue culture medium. Final concentration-250 mg./ml.

Fungizone (Amphotericin)

Amphotericin B 50 mg.

From: E. R. Squibb and Sons, Inc., New York, N.Y., 10022

Stock Solution

Dissolve contents of ampoule in 50 ml. sterile water for injection. Concentration-1000 mg./ml. Store at -20 C.

Working Solution

Add .25 ml. stock to 100 ml. culture medium Final concentration-2.5 mg./ml.

Explants for plating were taken from young plants growing near the shores of the heavily contaminated El Rio canal bordering the campus of Florida Atlantic University. Returned to the laboratory, these plants were surface sterilized using a combination of 10% Clorox and a few drops of Tween 80 for 15 minutes after having been cut into sizes convenient for handling. After three rinses in sterile distilled water, the plants were aseptically cut into thin (3 mm.) sections for plating. Specific tissues chosen from the plants were leaf, petiole, korm and root. The plates were observed for one week in a Percival environmental chamber programmed for 80°F. Timer settings for illumination allowed for a twelve hour day, beginning at 0600 and ending at 1800.

In a second effort, a detached leaf culture was employed preliminary to plating. Whole leaves from young plants taken from the same source were surface sterilized and placed in Petri dishes containing Murashige and Skoog's culture medium (Murashige and Skoog, 1962) as outlined in Table 3. The same proportions penicillin, streptomycin, and fungizone as shown previously were used.

After 48 hours, these leaves were removed from the growth chambers and aseptically sliced for plating on the "B" medium. Six squares of one centimeter in area were plated per dish. After plating, the dishes were returned to the controlled environment and observed for one week.

A third culture medium, Lamport's, was employed. (Lamport, 1964). None of the antibiotics previously used were added. Techniques and conditions were identical to those used in the "B" trial, the observation period being one week. An outline of Lamport's medium appears in Table 4.

TABLE 3

Chemical Composition and Dilutions for Murashige-Skoog Medium For Use in Tissue Culture of E. crassipes

Minerals (10X) Use 100 ml per liter of medium

NH_4NO_3	16.5 g
KNO_3	19.0 g
$CaCl_2.2H_2O$	4.4 g
$MgSO_4.7H_2O$	3.7 g
KH_2PO_4	1.7 g
H_3BO_4	62.0 mg
$MnSO_4.H_2O$	169.0 mg
$ZnSO_4.7H_2O$	106.0 mg
KI	8.3 mg
Water	1000 ml

Store at room temp., may be autoclaved.

Vitamins (10X) Use 10 ml per liter of medium

Glycine	20 mg
IAA	10 mg
Kinetin	0.5 mg
Myoinositol	1.0 g
Nicotinic Acid	5.0 mg
Pyrodoxine HCl	5.0 mg
Thiamine HCl	1.0 mg
Water	100 ml

Store in 10 ml lots in deep freeze.

Iron Use 5 ml per liter of medium

$Fe_2(SO4)_3.7H_2O$	0.557 g
Na_2EDTA	0.745 g
Water	100 ml

For one liter quantity:

Stock iron solution	5 ml
Stock minerals (10X)	100 ml
Stock vitamins (10X)	10 ml
Sucrose	30 g

Add distilled water to make final volume one liter. Mix thoroughly to dissovle.
Adjust pH to 6.0 - 6.1 before autoclaving.
Distribute into 100 ml lots and autoclave.
Final pH should be 5.7 - 5.8.

Chemical Composition and Preparation of Lamport's Medium for Use in Tissue Culture of E. Crassipes

TABLE 4

Lamport's medium stock solutions

Minerals (10X)

$Ca(NO_3)_2.4H_2O$	12.0 g
KNO_3	3.2 g
KCl	2.6 g
$MgSO_4.7H_2O$	15.0 g
Na_2SO_4	8.0 g
$NaH_2PO_4.H_2O$	.76 g
$MnSO_4.4H_2O$	.20 g
$ZnSO_4.7H_2O$	.12 g
H_3BO_3	.06 g
KI	.03 g

Dissolve in 4 liters distilled water, dispense, and autoclave.

Vitamins (10X)

Glycine	30 mg
Thiamine HCl	1 mg
Cysteine HCl	10 mg
Water	100 ml

Distribute in 10 ml lots and freeze.

Growth Regulators

Calcium pantothenate (Refrig.)	10 mg/100 ml
2,4-D (Room Temp.)	60 mg/20 ml

Iron

Na_2EDTA	80.0 mg
$Fe_2(SO_4)_3.7H_2O$	0.1 mg
Water	100 ml

Yields 1 mg/ml use 2.5/liter medium

TABLE 4 (Con't)

Preparation

For 1 liter quanity:

1. Into a large beaker add 2.5 ml stock iron sol'n.
2. Add 100 ml stock mineral sol'n.
3. Add 10 ml of stock vitamins (10X).
4. Add 1 ml calcium pantothenate stock and 0.2-2.0 ml of 2,4-D stock.
   NOTE: 2,4-D controls friability of culture. Use 2 ml stock for maximum friability.
5. Add 100 ml of coconut water (boiled, cooled, and filtered). Use fresh coconuts, taste for sweetness
6. Add 20 g sucrose.
7. Add water to one liter.
8. Adjust pH to 6.0 with KOH or HCL before autoclaving.
9. Final pH sould be 5.7-5.85.

If solid media is desired, add 10 g purified agar before step 8. At step 8, adjust to pH 6.2 (melt agar first, pH while warm) before autoclaving.

BONNY LOCH STUDY.

During field investigations seeking indigenous pathogens for noxious aquatic vegetation and their possible utilization as biological control agents, an anomalous condition was noted. A former limerock excavation which had filled with water and called Bonny Loch had been abandoned to a housing development. This body of water is located in the northwest section of Pompano Beach, Florida and is surrounded by similar excavations, some developed for housing and some still utilized for excavation of limerock. Neighboring canals, ditches, and lakes exhibited varying infestations with Eichhornia crassipes (water hyacinth) but none were present in Bonny Loch. No surface connection with neighboring bodies of water was located, however, due to the recreational utilization of the lake and public access through a boat ramp the probability is high that transport of plants or seeds from nearby bodies would occur. No hyacinths could be recalled by local residents, in the past and no previous control had been exercised. The lake, however, was heavily infested with Hydrilla verticillata (Florida elodea).

Two possibilities exist; one, no plants were introduced or two, conditions in the lake were unfavorable for hyacinth growth. If the latter were true, perhaps the factors could be determined and extended to heavily infested areas. To test this an experiment was designed to compare the growth of young Eichhornia crassipes plants in Bonny Loch with those in a waterway supporting luxuriant growth with rapid propagation.

A structure was constructed from galvanized screen to contain the plants and eliminate the possibility of introducing E. crassipes to the lake if case no. 1 above were true. Floatation of the structure was provided by plastic bottles affixed to the screen. Inflorescences which developed during the experiment were removed to preclude the possibility of fertile seed-set on this monecious plant. The waterway selected as the control was Lake Worth Drainage District Equalizing Canal No. 4, commonly referred to as the El Rio Canal, at a point 1 mile north of the 20th Street bridge in Boca Raton, Florida. This waterway regularly supports luxuriant growth of E. crassipes and is subjected to periodic herbicidal treatment.

Three small plants were selected for inclusion in each habitat. Routine observations of each group substantiated case 2 above.

PATHOGEN STUDIES (*ALTERNARIA* SP.)

A species of *Alternaria* fungus was isolated from a diseased water hyacinth collected from a drainage canal in south Palm Beach County, Florida. Isolation was performed by first surface sterilization of the leaf using 5.25% $NaHClO_3$. The leaf spots were then excised and homogenized in physiological saline and 1.0 ml. aliquots were plated onto several mycological agars at several temperatures. In order to satisfy the third of Koch's postulates, healthy hyacinths were inoculated by the fungus.

First, a 5 mm^2 agar block from a 10 day culture of *A. eichhorniae* was placed face down on the surface of a hyacinth leaf which had been scraped to break the epidermis and aid entry. Test plants were kept in high humidity for several days to enhance stomatal opening and promote fungal growth. Once the infection was established, the remaining portion of the agar block was removed.

The second method of infection was the injection of a saline suspension of the fungus into the petiole and leaf stem.

The third method, and most promising, was an aerosol spray of a spore suspension. Spores were obtained using a glass wool filtering technique in which heavily sporulating cultures were washed with buffered saline, filtered through sterile glass wool, and collected. A 5 sec. spray from an atomizer onto healthy plants was used for inoculation.

Serial sections of the infected leaf tissue were prepared and stained with safronin O and counter-stained with fast green. These sections were examined microscopically.

Nag Raj (1970) reported the existence of toxic metabolic products in a strain of *Alternaria* attacking hyacinths in India. The possibility of soluble toxic metabolite in our *Alternaria* was explored. *Alternaria* previously grown on malt extract agar was inoculated into 500 ml. of mycological broth and incubated for three days in a shaker at 25C. The resulting culture was filtered through Whatman filter paper using a Buchner filter. The solution was then filtered through a 0.45 Millipore membrane filter. The resulting solution was designated as soluble *Alternaria* metabolite and handled sterilely.

Three hyacinth leaves were scratched with a pin to break the epidermis. Sterile distilled water was placed on one, sterile mycological broth on another, and soluble *Alternaria* metabolite on the third.

A determination of host range of this fungus was attempted by inoculation onto various cash crops such as tomato, cabbage, green pepper, turnip, lima bean, stringbean, and eggplant.

In order to determine optimum growth conditions of this fungus, a series of studies was conducted utilizing commercially available media and media containing extracts of the water hyacinth.

Initial inoculations were made by placing 7 mm plugs (#5 Cork borer) from a 48 hr. culture in the center of petri plates (100 x 15 mm) containing 11 different media (Difico). These included the following:

V-8 juice agar (Browder, 1964), mycological agar, malt extract agar, potato dextrose agar, sabouraud agar, with dextrose sabouraud agar, with maltose bean pod agar, prune agar, starch agar, and corn meal agar with dextrose. Plates were inoculated in duplicate sets of two each and incubated at 25C. Growth in mm and amount of light absorbed were determined at 24 hr intervals for 7 days.

The amount of light absorbed by the culture was indicative of the culture density and amount of sporulation.

The % light absorbance was calculated using a white light source emmitting 2500 ft. cds. placed 40 cm above a Weston Illumination Meter, Model 4165. Color temperature and cosine correction factors have been incorporated.

Petri plates of each culture were placed on the photo cell and the difference in ft./candles of light between uninoculated control plates and the test plates was calculated as % absorbance.

Media containing water hyacinth extracts were prepared by Dr. Carlos Blasquez, University of Florida, IFAS-ARC, Imokolee, Florida. Two extraction procedures were employed; unboiled extraction with water and unboiled extraction with ethanol. Two grams of plant material, either leaves, stems or petioles, were minced and allowed to extract in 100 mls of solvent for 30 mins. The extraction solution was filtered sterilization and added to 900 mls of sterile water with 1.5% agar (Difco). Fifteen mls of medium was poured into each glass petri dish (100 x 15mm) and used as inoculation plates as described previously.

RESULTS

(Eichhornia crassipes)

GROWTH OF ENTIRE PLANT

Growth of individual plants in buckets had several inherent problems. 1) wind damage to leaves and petioles; 2) high temperature of water in buckets; 3) rapid depletion of nutrients in small amount of water in buckets; 4) rapid loss of water volume through evaporation and transpiration. This method did have the advantage of easy transport from growth area to laboratory.

Growth in the wading ponds alleviated certain problems encountered in bucket studies. Wind damage was reduced by the increased number of plants - outer plants shielded the plants toward the center of the group. Upon establishment of Eichhornia in the ponds, organic debris collected thereby cycling much of the necessary nutrients. Plants were susceptible to damage by aphids; however, this could be controlled by the addition of Isotox. For high temperature problems, slat houses were constructed to cut direct sunlight to 50% effectively lowering water temperature.

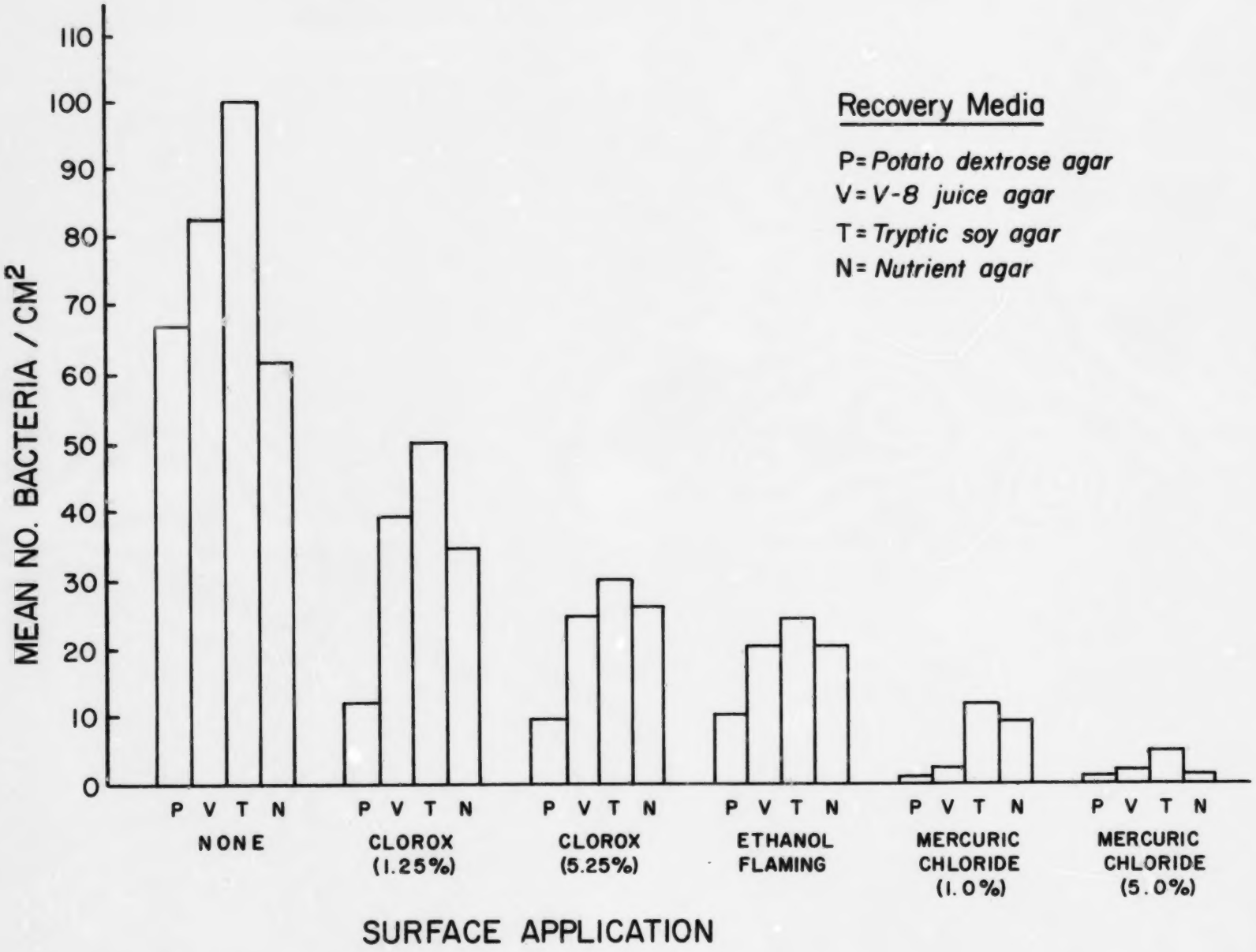
Growth of Eichhornia in environmental chamber proved to be less sensitive than growth of Hydrilla. Experiments were designed to establish optimal growth conditions. Growth conditions proved to be rather broad in nature as to range of temperatures, light intensity and nutrients.

Growth was good at a temperature range of from 20 to 27C and light intensity of from 1400 to 3000 ft. candles. Any complete fertilizers such as Hoagland's or a 6-6-6 type promoted good growth. It was found that Eichhornia grows well in extremely high nutrient situations. The growth of Eichhornia presents no major problems in an outdoor or controlled environment.

SURFACE STERILIZATION

The arithmetic mean of the number of bacteria which were cultured after 24 hrs. from each leaf is presented in Figure 2. A decline in the total number resulted from all treatments, as compared to the untreated control group. The highest number of colonies were recovered from leaves treated with 1.25% Clorox and the least number recovered from 5.0% mercuric chloride with an intermediate number recovered from 5.0% ethanol flaming. Upon reincubation of the plates for a total of 48 hours numerous mucoid colonies had converged, rendering counts of discreet colonies difficult, therefore no quantitative bacterial counts were performed after 24 hours. There was no attempt to identify those bacteria which were isolated but a predominance of gram negative forms was observed.

FIG. 2 Bacterial recovery from E. crassipes leaves treated with various surface sterilants.


MEAN NO. BACTERIA / CM²
110
100
90
80
70
60
50
40
30
20
10
0
Recovery Media
P = Potato dextrose agar
V = V-8 juice agar
T = Tryptic soy agar
N = Nutrient agar
P V T N
NONE
CLOROX (1.25%)
CLOROX (5.25%)
ETHANOL FLAMING
MERCURIC CHLORIDE (1.0%)
MERCURIC CHLORIDE (5.0%)
SURFACE APPLICATION

The fungal counts were performed from potato dextrose agar after 48 hours incubation by counting all colonies present on each plate (Table 5). The data presented is the total number of colonies present on twenty samples. The ambient flora was reduced by all applications with the lowest counts resulting from the ethanol flaming and the highest from the Clorox treatments. Incubation of the plates beyond 48 hours resulted in overgrowth of many plates by fungal hyphae rendering invalid any further quantitative examination. No attempts were made to identify those fungi which were cultured.

DETACHED LEAF CULTURE

1. Manipulative investigations.

Sterile plastic petri dishes were difficult to wet and caused rounding of the liquid culture medium which presented a non-flat surface on which to float the leaf. All five leaves cultured in plastic dishes exhibited slight chlorosis following two weeks' culture which progressed into the third week.

Glass petri dishes were uniformly wetted and produced a flat medium surface on which to float the leaf. All five leaves cultured in glass dishes exhibited normal color after three weeks' culture.

Tilted glass petri dishes were not satisfactory because they were more precarious than flat dishes. This necessitated a greater spacing between culture vessels than with flat plates to avoid upsetting neighboring cultures whenever the leaves were tended. The leaves cultured three weeks in tilted petri plates exhibited slight general chlorosis; an appearance comparable to the leaves cultured in plastic dishes.

The moistened filter paper failed to maintain the turgor of the leaves. A flaccid texture and mottled chlorosis resulted after one week of culture. The point of excision from the petiole became necrotic without apparent growth of microorganisms. This necrosis remained localized within a few millimeters of the excision throughout the first week. The cultures were discarded after one week because of the rapid chlorosis as compared to the three other techniques.

Those leaves which received pretreatment, either in Tween 80, Clorox or distilled water did not significantly differ in appearance after three weeks' culture from those leaves which received no pretreatment. The culture media of the leaves which received no treatment, however, contained sand and miscellaneous debris in three of the five plates whereas those leaves which received pretreatment showed no detritus in the culture medium.

TABLE 5. Fungal colonies recovered from *Eichhornia crassipes* leaves after treatment with surface sterilants.

Surface application	Fungal colonies per 20 samples
None	76
1.25% Clorox	31
5.25% Clorox	26
Ethanol flame	6
1.0% mercuric chloride	20
5.0% mercuric chloride	16

2. Preliminary media study.

The results of the preliminary media study are presented in Table 6. The table is arranged in descending order of observed senescence. No appreciable difference was detected in the results produced by benzimidazole and by kinetin. All media which contained sucrose - viz. sucrose 2%, benzimidazole in 2% sucrose and kinetin in 2% sucrose - exhibited turbidity which was indicative of the growth of contaminating microorganisms. This turbidity did not appear in the media of all these cultures, rather in two of the five kinetin in 2% sucrose and three of the five plates cultured in the other media. Hoagland's solution was unable to sustain the green color of the leaves beyond the first week. Both tap water and distilled water showed advanced chlorosis by the end of the first week with total chlorosis occurring between the second and third weeks.

3. Biochemical comparison.

The light source at the initiation of this phase of the research was composed of two soft white and two Gro-Lux Flourescent tubes. During the first week of culture, chlorosis of the outer portions of the blade occurred at a much more rapid rate than that of the rest of the leaf. After extinguishing the Gro-Lux bulbs no further areas of rapid chlorosis developed.

The color values of these leaves are presented in Table 7 as a representation of the overall condition of the remaining leaves. In this evaluation the attempt was to establish minute differences between the ability of the various treatments to delay chlorosis, therefore, the pluses were employed to establish these differences. The arrangement of this table is also one of descending order of observed senescence.

No apparent differences were noted between leaves cultured on the two concentrations of benzimidazole which were employed. Leaves cultured on both concentrations were capable of retaining a deep rich green appearance through the second week of culture with a slowly developing yellowish tint throughout the remainder of the four weeks, resulting in a predominance of green as compared to yellow at the termination. Kinetin at the 50 ppm condazole. At a kinetin concentration of 100 ppm, however, a more rapid rate of yellowing occurred in a non-uniform fashion throughout the blade producing a mottled-green appearance after three weeks of culture.

TABLE 6. Color value[a] of detached Eichhornia crassipes leaves maintained on the indicated medium. Preliminary medium selection.

Medium	weeks				
	0	1	2	3	4
Benzimidazole (50 ppm)	5	5	4	3	3
Kinetin (50 ppm)	5	5	4	3	3
Sucrose (2% w/v)	5	4	3[b]	2[b]	1[b]
Benzimidazole (50 ppm in 2% Sucrose)	5	5	3[b]	2[b]	1[b]
Kinetin (50 ppm in 2% Sucrose)	5	5	3[b]	2[b]	1[b]
Hoagland Solution	5	4	3	2	1
Tap Water	5	3	2	1	1
Distilled Water	5	3	2	1	1

a. Color value of normal leaves rated as 5 and leaves devoid of green as 1.

b. Solution becoming turbid as a result of contaminating growth.

TABLE 7. Color value[a] of detached Eichhornia crassipes leaves maintained on the indicated medium. Biochmeical studies.

Medium	Weeks				
	0	1	2	3	4
Benzimidazole (50 ppm)	5	5	4	3+	3
Benzimidazole (100 ppm)	5	5	4	3+	3
Kinetin (50 ppm)	5	5	4	3+	3
Kinetin (100 ppm)	5	5	4	3	
Kinetin (50 ppm) - Benzimidazole (50 ppm)	5	5	4	3	2+
Distilled Water	5	3	2	1	1

a. Color value of normal leaves rated as 5 and leaves devoid of green as 1

The method of sampling produced a decline in the number of leaves available each week from which to select the optimal leaves for biochemical determination. Although the experiment was initiated with 21 leaves for each medium and by the fourth week, at a depletion rate of three leaves per week, 12 leaves remained from which to select. The remaining population was not indicative of the overall trends noted. Two extraneous agents increased the senescence of the remaining leaves. One of these agents is an aphid, Rhopalosiphum nymphaeae (specimens identified by the U.S.D.A. Agricultural Research Service, Beltsville, Maryland). This organism oviposits in the leaves of E. crassipes during the Spring and Summer; then upon maturation to the nymph produces lesions which enhance the death rate of the leaves. The presence of the aphids in conjunction with the damage caused by the Gro-Lux light rendered insignificant any sampling of the kinetin 100 ppm solution past the third week of the experiment and all other cultures past the fourth week of treatment. The rate of decline of the leaves cultured in the mixture of kinetin and benzimidazole at 50 ppm concentration of each exhibited a pattern of decline similar to the kinetin 100 ppm. Luxuriant green appearance was sustained by both media through the second week with slight decline in color until the third to fourth week. After four weeks of culture, the leaves exhibited a heavily mottled yellowing which was further advanced than the previously mentioned agents at four weeks.

Culture of the leaves on distilled water produced almost total chlorosis by the second week and total lack of vitality by the third week. At the time of sampling on the third week, the medium was tinted brown from leached chemicals and dead tissue. The leaves were fragile rendering handling and sampling difficult.

The inside diameter of the number seven cork borer measured 10.4 mm. Each disc, therefore, had a surface area of 169.810 $mm.^2$ and the total surface area sampled for each leaf (15 discs totaled 2547.150 $mm.^2$). The mean wet weight of fifteen discs from each of five test leaves is 155 $\pm$ 23.3 mg. at a 95% level of confidence.

B. Carbohydrate levels.

The carbohydrate levels obtained from the various samples are presented in Tables 8 and 9. Comparison of the arithmetic mean of the samples obtained for each week (Figs. 3 & 4) illustrates a decline in carbohydrate levels throughout the experimental period.

TABLE 8. Carbohydrate levels (ug/ml of aqueous extract), with the mean and standard deviation, for detached E. crassipes leaves maintained on the indicated medium.

Culture Medium	Weekly Sample #	Weeks 0	1	2	3	4
Initial leaves	1	315				
	2	343				
	3	332				
	4	354				
	$\bar{x}$	336				
	s	14.7				
Distilled Water	1		210	113	82	
	2		175	94	68	
	3		176	108	—a	
	$\bar{x}$		187	105	75	
	s		17.2	8.0	7.0	
Kinetin 50 ppm	1		175	205	80	30
	2		134	125	105	60
	3		159	95	85	100
	$\bar{x}$		156	141	90	63
	s		16.8	46.0	12.2	29.1
Kinetin 100 ppm	1		148	160	70	
	2		124	133	75	
	3		154	118	45	
	$\bar{x}$		142	137	63	
	s		12.9	17.4	13.1	

a. Leaf was too fragile to sample due to advanced senescence.

TABLE 9. Carbohydrate levels (ug/ml of aqueous extract), with the mean and standard deviation, for detached E. crassipes leaves maintained on the indicated medium.

Culture Medium	Weekly Sample #	Weeks 0	1	2	3	4
Benzimidazole-Kinetin, 50 ppm each	1		275	105	145	60
	2		275	120	105	75
	3		250	200	85	120
	$\bar{x}$		266	141	111	85
	s		11.8	41.0	24.9	25.4
Benzimidazole 50 ppm	1		337	200	100	75
	2		273	225	110	105
	3		260	275	125	115
	$\bar{x}$		290	200	111	98
	s		33.6	20.4	10.3	17.0
Benzimidazole 100 ppm	1		253	170	99	55
	2		284	175	105	120
	3		258	280	120	105
	$\bar{x}$		265	208	108	93
	s		12.2	50.1	8.9	27.7

TABLE 10. Nitrogen levels (ug/ml of aqueous extract) of detached E. crassipes leaves maintained on the indicated medium, including the arithmetic mean, standard deviation, and ratio of soluble nitrogen to total nitrogen.

		Weeks									
		0		1		2		3		4	
Culture Medium	Weekly Sample #	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.
Initial Leaves	1	267	126								
	2	290	178								
	3	281	150								
	4	295	163								
	$\bar{x}$	283	154								
	s	10.6	19.0								
	Ta	436									
	S/T[b]	0.35									
Distilled Water	1			388	207	95	355	—[c]	462		
	2			341	155	70	311	—[c]	442		
	3			332	227	76	328	—[d]	—[d]		
	$\bar{x}$			353	196	80	331				
	s			24.5	30.3	10.6	18.0				
	Ta			549		411					
	S/T[b]			0.356		0.805					
Kinetin 50 ppm	1			273	244	137	215	150	422	168	402
	2			210	203	120	312	160	385	154	477
	3			245	225	175	270	145	450	136	437
	$\bar{x}$			242	224	144	266	152	419	152	439
	s			25.7	16.7	25.2	39.7	6.8	26.6	12.3	28.0
	Ta			466		410		571		591	
	S/T[b]			0.48		0.649		0.735		0.743	

a. total nitrogen = $\bar{x}$ soluble nitrogen + $\bar{x}$ insoluble nitrogen
b. ratio of soluble nitrogen to total nitrogen
c. concentration below the range of this test
d. leaf was too fragile to sample due to advanced senescence

TABLE 11. Nitrogen levels (ug/ml of aqueous extract) of detached E. Crassipes leaves maintained on the indicated medium, including the arithmetic mean, standard deviation, and the ratio of soluble nitrogen to total nitrogen.

		Weeks							
		0		1		2		3		4	
Culture Medium	Weekly Sample #	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.	Sol.
Kinetin 100 ppm	1			224	171	110	314	165	408		
	2			193	183	180	300	140	460		
	3			212	217	88	280	190	425		
	$\bar{x}$			209	190	126	298	165	431		
	s			12.8	19.0	14.1	14.0	20.4	21.6		
	T[a]			399		424		596			
	S/T[b]			0.476		0.703		0.723			
Benzimidazole-Kinetin, 50 ppm each	1			246	214	283	268	85	297	75	387
	2			307	176	329	278	118	342	90	391
	3			254	232	273	224	115	333	87	443
	$\bar{x}$			269	207	295	257	106	324	84	407
	s			27.0	22.6	24.2	23.4	14.8	18.5	6.5	25.5
	T[a]			476		552		430		491	
	S/T[b]			0.433		0.464		0.754		0.83	
Benzimidazole 50 ppm	1			112	212	224	252	60	315	122	384
	2			140	253	189	224	80	227	97	357
	3			153	218	152	302	140	385	87	355
	$\bar{x}$			135	227	221	259	93	309	101	366
	s			17.0	18.0	25.7	31.0	32.0	20.0	15.1	13.2
	T[a]			362		480		402		467	
	S/T[b]			0.627		0.541		0.768		0.784	
Benzimidazole 100 ppm	1			189	157	364	287	70	297	67	452
	2			140	117	350	339	70	262	78	495
	3			168	132	434	270	100	385	74	484
	$\bar{x}$			166	135	382	299	80	314	73	477
	s			20.0	16.5	36.7	29.3	14.1	51.6	4.5	18.2
	T[a]			301		581		394		550	
	S/T[b]			0.448		0.439		0.796		0.868	

a. total nitrogen = $\bar{x}$ soluble nitrogen + $\bar{x}$ insoluble nitrogen
b. ratio of soluble nitrogen to total nitrogen

FIG. 3 Carbohydrate levels (per ml of aqueous extract), measured as total carbohydrate, of detached E. crassipes leaves maintained on the indicated medium.

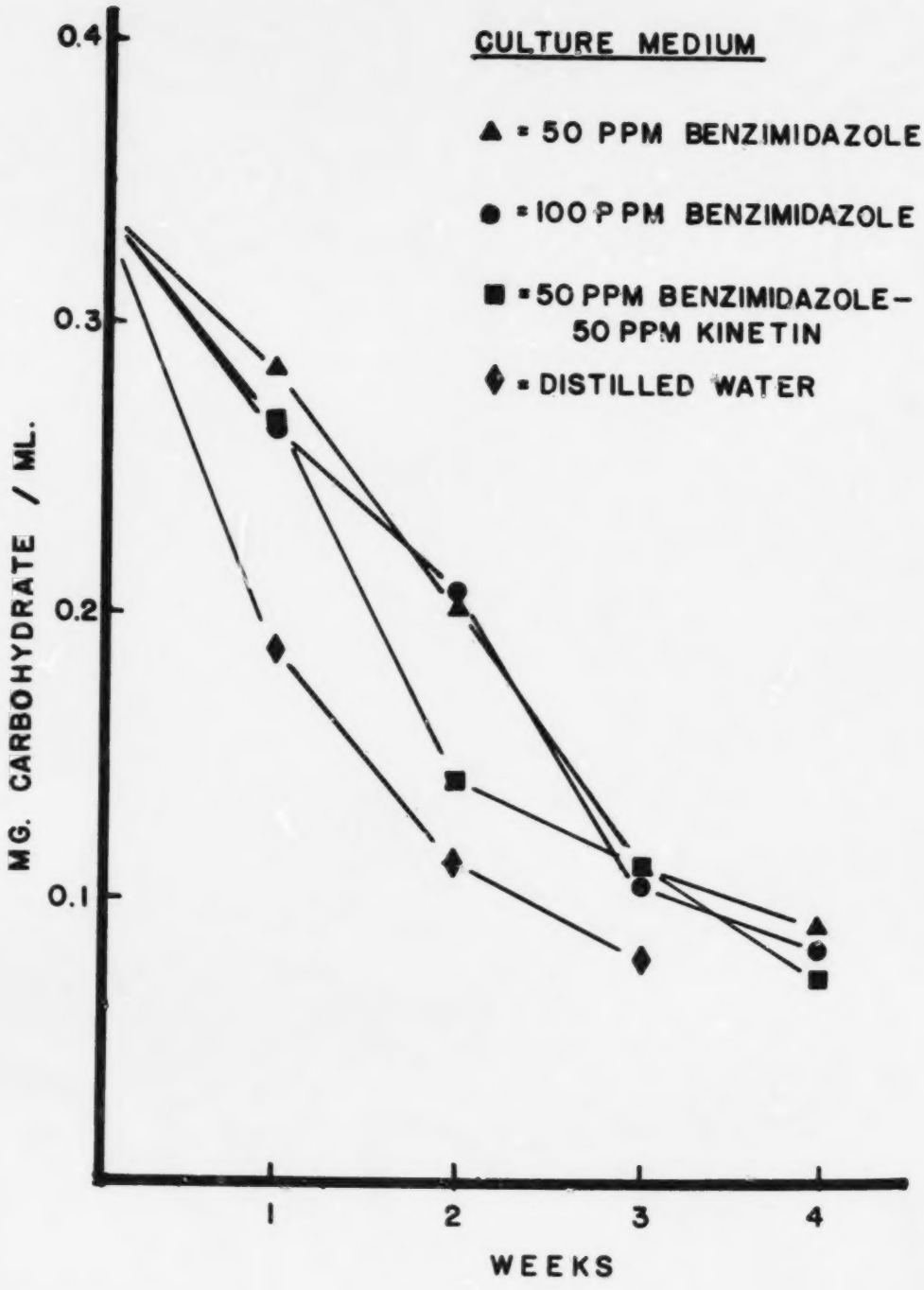
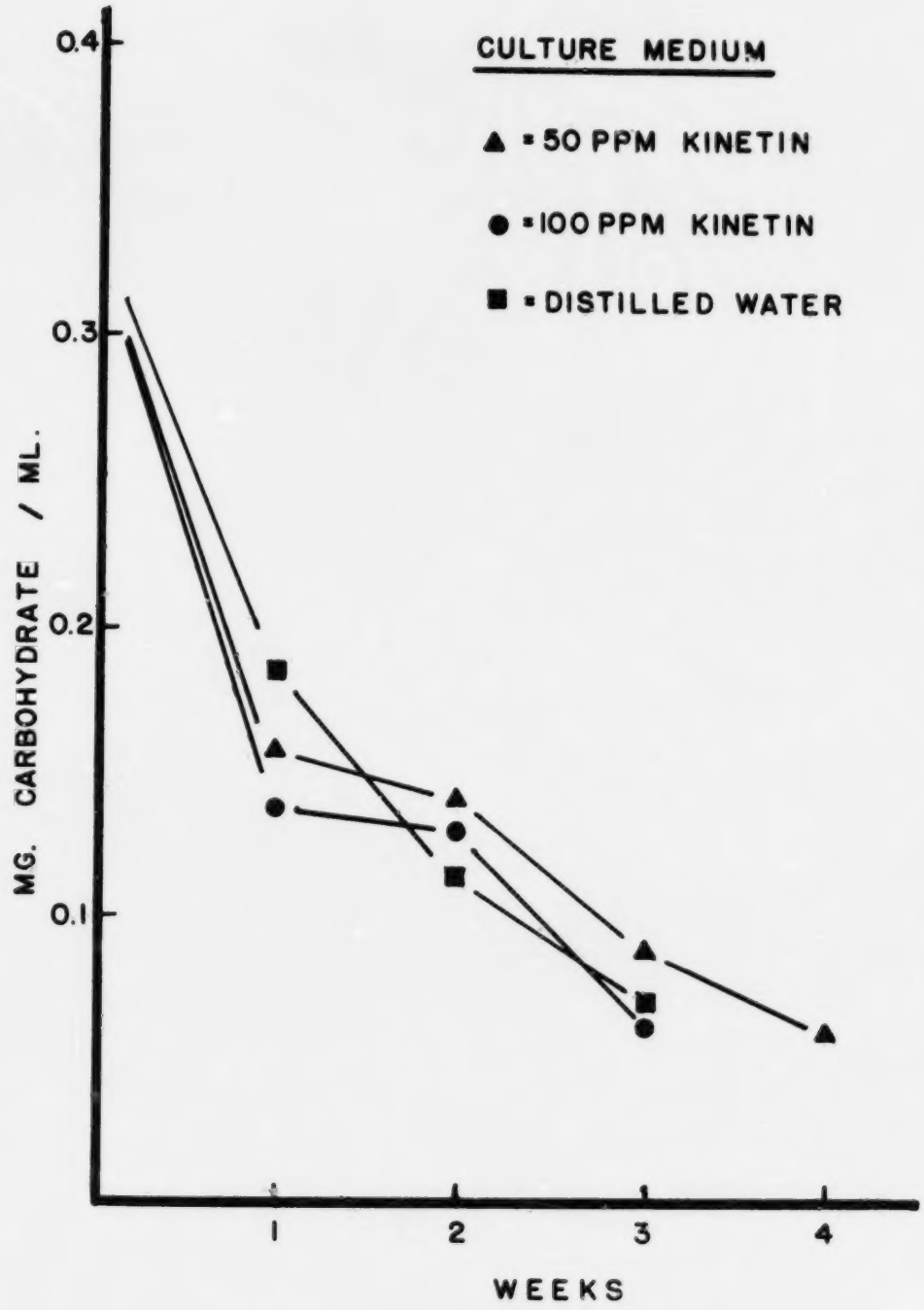

CULTURE MEDIUM
▲ = 50 PPM BENZIMIDAZOLE
● = 100 PPM BENZIMIDAZOLE
■ = 50 PPM BENZIMIDAZOLE – 50 PPM KINETIN
♦ = DISTILLED WATER
MG. CARBOHYDRATE / ML.
0.4
0.3
0.2
0.1
1
2
3
4
WEEKS

FIG. 4. Carbohydrate levels (per ml of aqueous extract), measured as total carbohydrate, of detached E. crassipes leaves maintained on the indicated medium.


CULTURE MEDIUM
▲ = 50 PPM KINETIN
● = 100 PPM KINETIN
■ = DISTILLED WATER
MG. CARBOHYDRATE / ML.
0.4
0.3
0.2
0.1
1
2
3
4
WEEKS

Comparison of the two concentrations of benzimidazole and the mixture of benzimidazole and kinetin, Fig. 3, shows little deviation between the respective levels of each for the term of the experiment. The mixture of the agents exhibited a more rapid decline than the two concentrations of benzimidazole after two weeks to a level of 0.135 mg. carbohydrate per ml., otherwise the carbohydrate levels compared favorably to that for benzimidazole. Evaluating these three agents as representations of the same reaction, a decline from the original level of 0.33 mg/ml. of aqueous extract (10 ml. total) is noted to 0.29 mg/ml. the first week, 0.21 mg/ml. the second, 0.11 mg/ml. the third, and 0.10 mg/ml. the fourth and last week. The levels for all three agents were consistently higher than those for leaves cultured on distilled water.

The carbohydrate levels for the two concentrations of kinetin followed the patterns of degradation (Fig. 4) similar to each other and differed only slightly from the decline of leaves cultured on distilled water. Leaves cultured on the two concentrations of kinetin exhibited a decline from the initial level of 0.33 mg/ml. to 0.15 mg/ml. the first week, with little change the second, a decline to 0.09 mg/ml. the third week and little additional change with the only concentration sampled in the fourth week.

The distilled water control exhibited a decline to 0.19 mg/ml. the first week, 0.12 mg/ml. the second, and finally to a concentration of 0.09 mg/ml. on the third week of culture Fig. 4). The deterioration of the leaves prohibited sampling past the third week of culture.

C. Nitrogen levels.

The soluble and insoluble nitrogen levels are presented in Tables 10 and 11. Figures 5 and 6 depict the ratio of soluble nitrogen to total nitrogen, computed as follows:

Total nitrogen = Soluble nitrogen + Insoluble nitrogen
S/T Ratio = Soluble nitrogen/Total nitrogen

The ratio of soluble nitrogen to total nitrogen in leaves cultured on 50 ppm benzimidazole increased from an initial value of 0.35 to 0.62 the first week followed by a decline to 0.56 the second week, then a further increase to 0.76 the third week with little additional change the fourth week (Fig. 5). Little difference was noted between the values obtained for leaves cultured on benzimidazole 100 ppm and benzimidazole-kinetin solution (Fig. 5), both indicated slight change in values through the second week of culture with a subsequent large increase the third week followed by little change thereafter. Comparison of the nitrogen values of these two media with those obtained for leaves maintained on distilled water indicates a substantial difference between these during the period between the first

FIG. 5. Nitrogen levels (per ml of aqueous extract) of detached E. crassipes leaves maintained on the indicated medium. Levels are expressed as the ratio of water soluble nitrogen of the alcohol soluble fraction of leaf extract to total nitrogen.

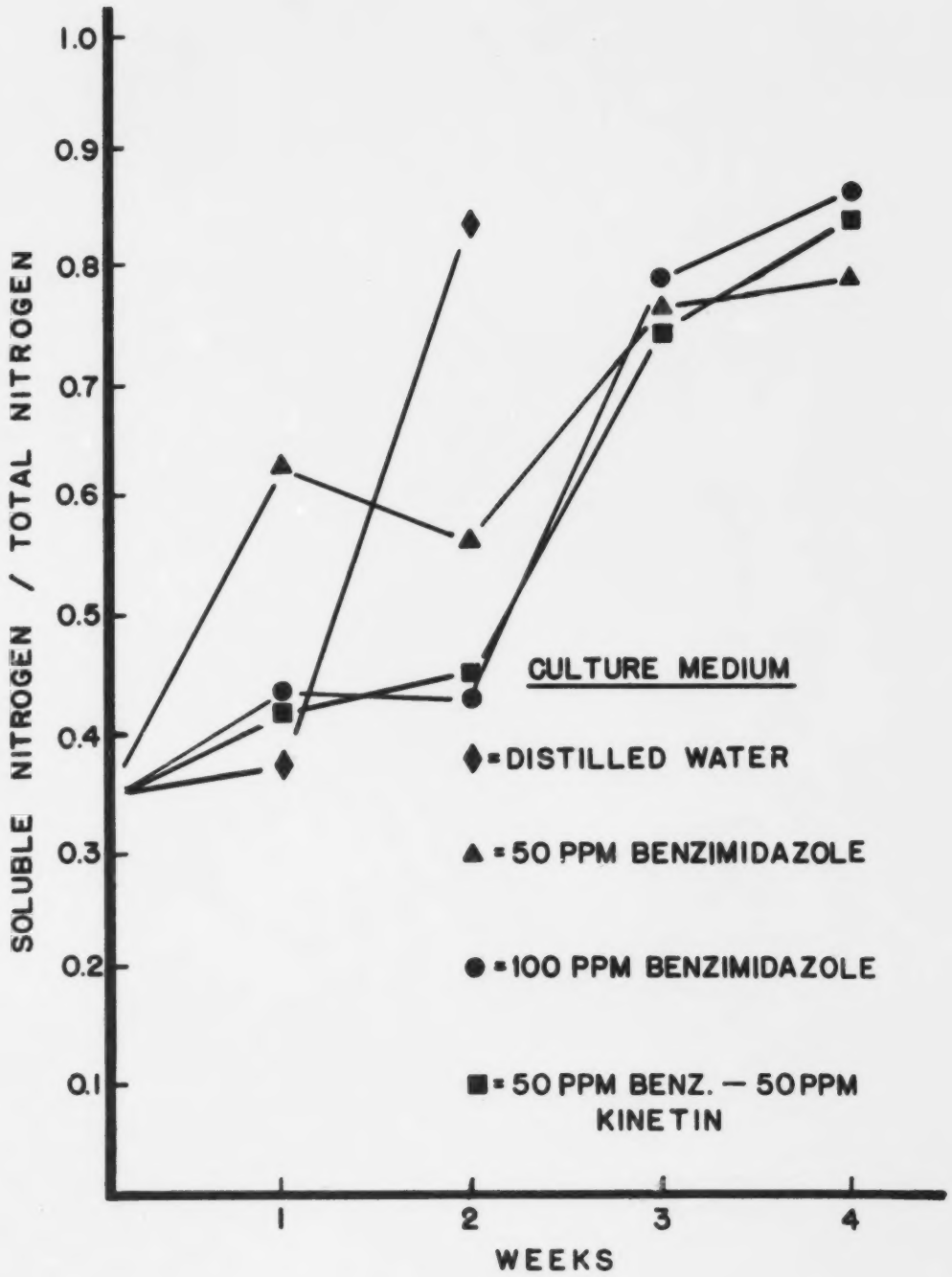
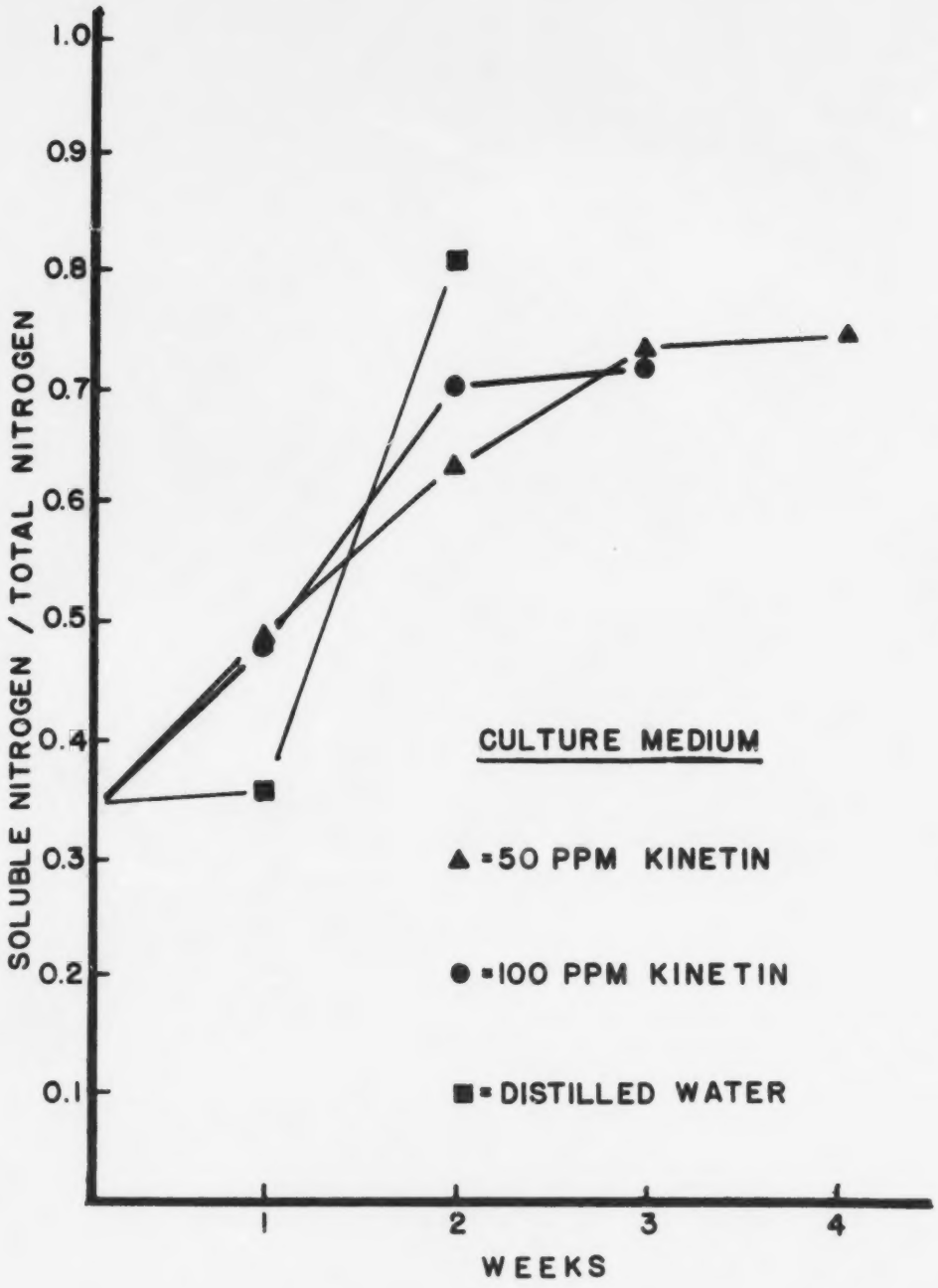

SOLUBLE NITROGEN / TOTAL NITROGEN
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
1
2
3
4
WEEKS
CULTURE MEDIUM
♦ = DISTILLED WATER
▲ = 50 PPM BENZIMIDAZOLE
● = 100 PPM BENZIMIDAZOLE
■ = 50 PPM BENZ. — 50 PPM KINETIN

FIG. 6. Nitrogen levels (per ml of aqueous extract) of detached E. crassipes leaves maintained on the indicated medium. Levels are expressed as the ratio of water soluble nitrogen of the alcohol soluble fraction of leaf extract to total nitrogen.


1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
SOLUBLE NITROGEN / TOTAL NITROGEN
1
2
3
4
WEEKS
CULTURE MEDIUM
▲ = 50 PPM KINETIN
● = 100 PPM KINETIN
■ = DISTILLED WATER

and second week of culture. The rapid change of distilled water cultured leaves from a ration of 0.356 after one week to 0.805 after two weeks' culture is not reflected in leaves cultured on benzimidazole-kinetin mixture.

Nitrogen ratios of leaves cultured on 50 ppm kinetin showed uniform increase throughout the first three weeks of culture at which time a plateau was attained near a ratio of 0.71 followed by little change in the fourth week (Fig. 6). Leaves cultured on kinetin 100 ppm reached a plateau at approximately a ration of 0.7 following two weeks' culture or one week earlier than leaves cultured on kinetin 50 ppm.

The distilled water control exhibited little change during the first week of culture followed by a rapid increase to a level of 0.805 after 2 weeks' culture. Samples of the leaves in distilled water taken after three weeks of culture had levels of insoluble nitrogen which were undetectable by the method employed. Computation of a ratio for the third week of culture would, therefore, yield a value of 1.0. This value, however, is not accurate since insoluble nitrogen levels were not truly zero. No ratio was entered for the third week of culture on distilled water.

TISSUE CULTURE OF PLANT PARTS.

It was noted during observation that Eichhornia explants had a tendency to dry out on the surface of the media rapidly. To remedy this problem, a suspension culture of "B" medium might be tried by deleting the agar during preparation.

In every case, without exception, fungicide contamination prohibited proliferation. Even after antibiotic detached leaf culture, contamination was extensive. It was encouraging to note however that bacterial contamination was minimal, indicating successful control with the use of penicillin and streptomycin. The fungus problem might be controlled by doubling the concentration of fungizone, but a final concentration of over 5.0 mg/ml. might be inhibitory to culture growth. If fungal growth cannot be controlled by antibiotics, sterile plants are the only alternative as a source for contaminant-free explants. Surface sterilized seeds grown under septic conditions should prove satisfactory.

By contrast, the control group had substantially increased in both size and number. The plants by the end of four months had breached the confines of the cage and increased in number to 10. No quantitative comparison is available due to herbicidal treatment of the control along with the canal but generally a 5 to 6 fold increase in size occureed during the experiment whereas the Bonny Loch group exhibited no appreciable increase in size.

The environmental variables, i.e., radiation, photoperiod, water temperature, air temperature, rainfall, and humidity, were relatively the same for each group throughout the experiment. The major uncontrollable variable was that of nutrients and their concentration.

PATHOGENIC STUDIES (ALTERNARIA SP.)

The data indicate that the Alternaria isolate grew substantially well on all media tested, with the exception of prune agar. (Figs. 7, 8, 9, and 10). There appeared to be little if any significant differences in either the colony size or rate of growth during the 7 day incubation period. The most noticeable difference was in amount of sporulation on corn meal agar, bean pod agar, and prune agar (Fig. 8). Light absorbance reached a maximum of 75% or 1/4 less than all other media even though they supported equally good mycelial growth. Maximum growth, both mycelial and sporulation, was obtained with PDA and for this reason the culture has been maintained on this medium.

The media containing water hyacinth plant part extracts showed similar hyphal growth. With water as the extract solvent (Fig. 11) the leaf medium provided the best growth. With ethanol as the solvent, however, the meristematic corm area gave optimum growth (Fig. 12). Although % light absorbance is not indicated on the graphs, sporulation on all extract test media was very poor. Maximum light absorbance was 26% with the average being 21%.

Since the best growth occurred on potato dextrose agar at 25C it has subsequently been maintained on this medium. Thus, the first two of Koch's postulates were observed. That is (1) the organism must be present in the diseased host; and (2) it must be isolated from that host and grown in pure culture.

The laboratory induced disease is characterized by the development of a general chlorosis 2-3 mm in diameter at the initial site of inoculation which, with time, increases in size and darkens forming a classical leaf spot (Fig. 13). Shotting may occur following a necrosis of the leaf and death of the leaf and petiole usually result within two weeks (Fig. 14). The symptomology of these infections is in keeping with the symptoms of the disease as originally observed.

Several days after inoculation, the tissue adjoining the agar block turned brown and was surrounded by a yellow halo which enlarged with time. Once infection was established, the remaining portion of the agar block was removed, revealing an enlarging area of necrosis which resembled the initially observed leaf spot from which the original isolation was made. Alternaria was subcultured from the margin of the necrotic area on the infected leaf.

Studies of both scraped and non-scraped leaves revealed no significant differences in time or nature of infection.

Infected leaves typically produce leaf spots from which Alternaria can be re-isolated. A fine portion of infected leaf placed on a normal leaf will transmit the infection and re-isolation of Alternaria from the second leaf has been accomplished. Agar blocks placed on the petiole yielded necrotic areas similar to the leaf spots, re-isolation is also possible. Agar blocks placed in the root system, under water, gave no sign of infection.

Spore suspensions of Alternaria placed on both scraped and non-scraped leaves produced typical leaf spots. Spores injected into the petiole caused an outside browning of the petiole within several days. Examination of the inside of the petiole revealed mushy and rotten tissue from which Alternaria was re-isolated. Injection of spores into the corm or meristematic area caused no visible change in the plant or localized corm tissue. Similarly, introduction of spores into the rhizosphere caused no changes. A 5 sec. spray of spore suspension from an atomizer onto healthy, uninjured plants resulted in death of 50% of the leaves within 10 days. In all instances, re-isolation of Alternaria was accomplished.

Examination of the serial sections revealed that the fungal mycelia occurred both inter- and intra-cellularly. Hyphae were branched, septate and approximately 1-2 microns wide. Conidia appeared on the upper surface of the leaf, often arising from the stomata (Figs. 15, 16 and 17).

In the Alternaria soluble metabolite studies the first two leaves showed no necrosis and scratched spots appeared to heal leaving only inactive scars. The soluble Alternaria metabolite leaf within 24 hours developed an area of necrosis over the entire treated area (Fig. 18). The same three substances were injected into the petiole. No reaction from the first two was observed; however, the soluble metabolite caused brown mushy tissue to be produced along the periphery of the wound and injection area.

In the host range studies, no takes have been observed on any plant except the water hyacinth. It is important to note that infectivity is limited to the leaves and petioles. Thus far, we have been unable to establish infection in the meristematic corm area of the hyacinth. Until this can be accomplished, the rapid vegetative growth of E. crassipes can produce new leaves at a greater rate than the Alternaria can infect.

Characterization of the fungus has shown it to belong to the order Moniliales or imperfect of the class Ascomycetes. It produces hyphae which are both branched and septate approxi-

mately 1.5 mm wide. Conidia are borne on conidiophores which arise from the hyphae. They are typically club-shaped (Fig. 20) and occur in both branched and unbranched chains (Fig. 19). They range in size from 13 mm to 61 mm in length and 13 to 22.5 mm wide with 1-2 longitudinal or oblique septa and 4-10 transverse septa. When grown on malt extract medium it produces a slightly diffusable organic pigment.

FIG. 7. Growth rate studies of Alternaria on commercial media showing colony size and light absorption.

▲ ——— V-8 juice agar

● ——— Sabouraud agar w/maltose

■ ——— Starch agar

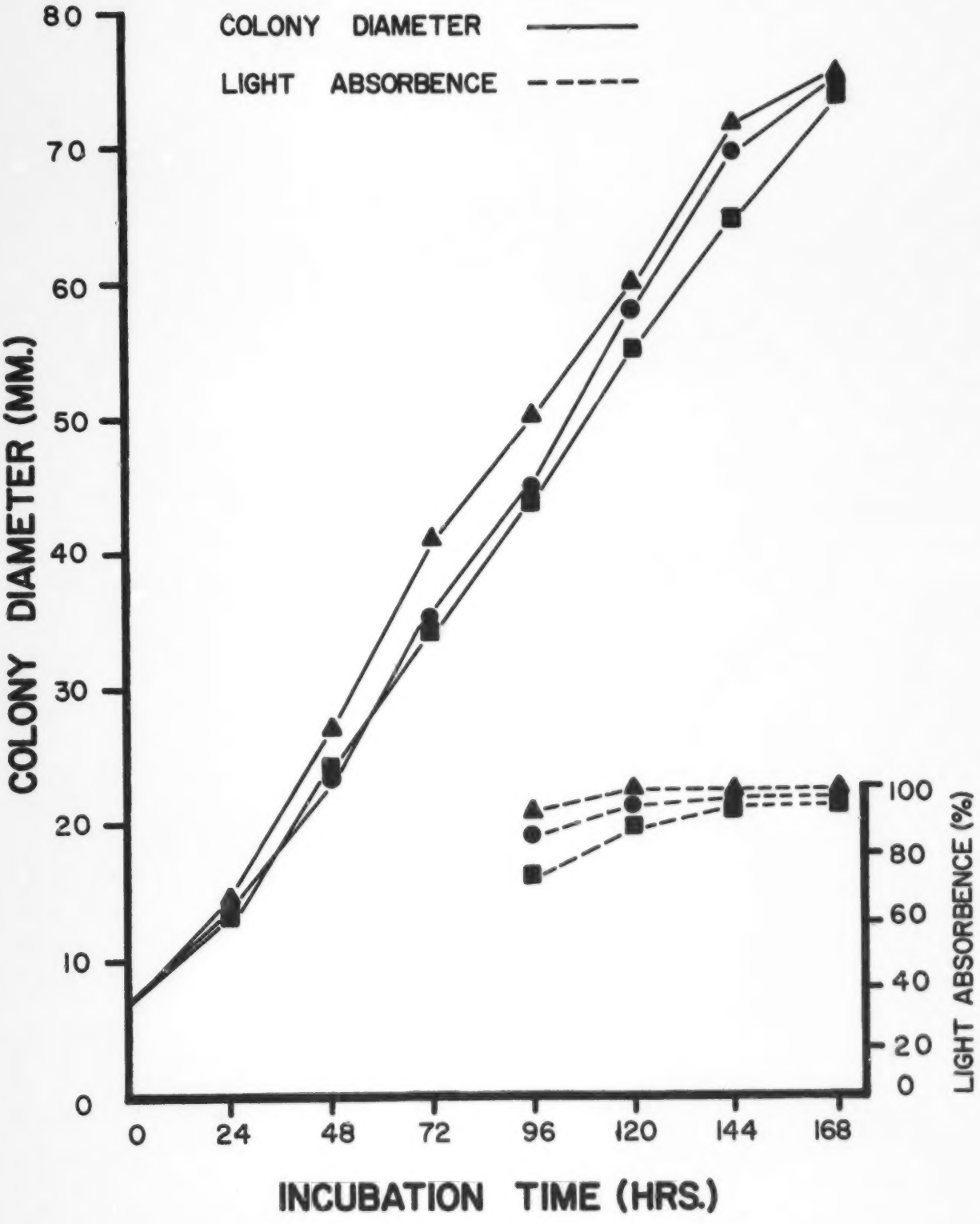
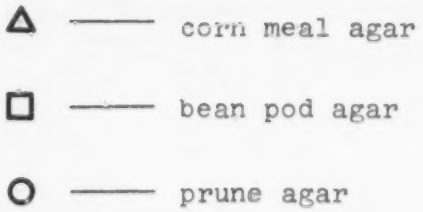

COLONY DIAMETER
LIGHT ABSORBENCE
COLONY DIAMETER (MM.)
0
10
20
30
40
50
60
70
80
0
24
48
72
96
120
144
168
INCUBATION TIME (HRS.)
LIGHT ABSORBENCE (%)
0
20
40
60
80
100

FIG. 8. Growth rate studies of Alternaria on commercial media showing colony size and light absorption.



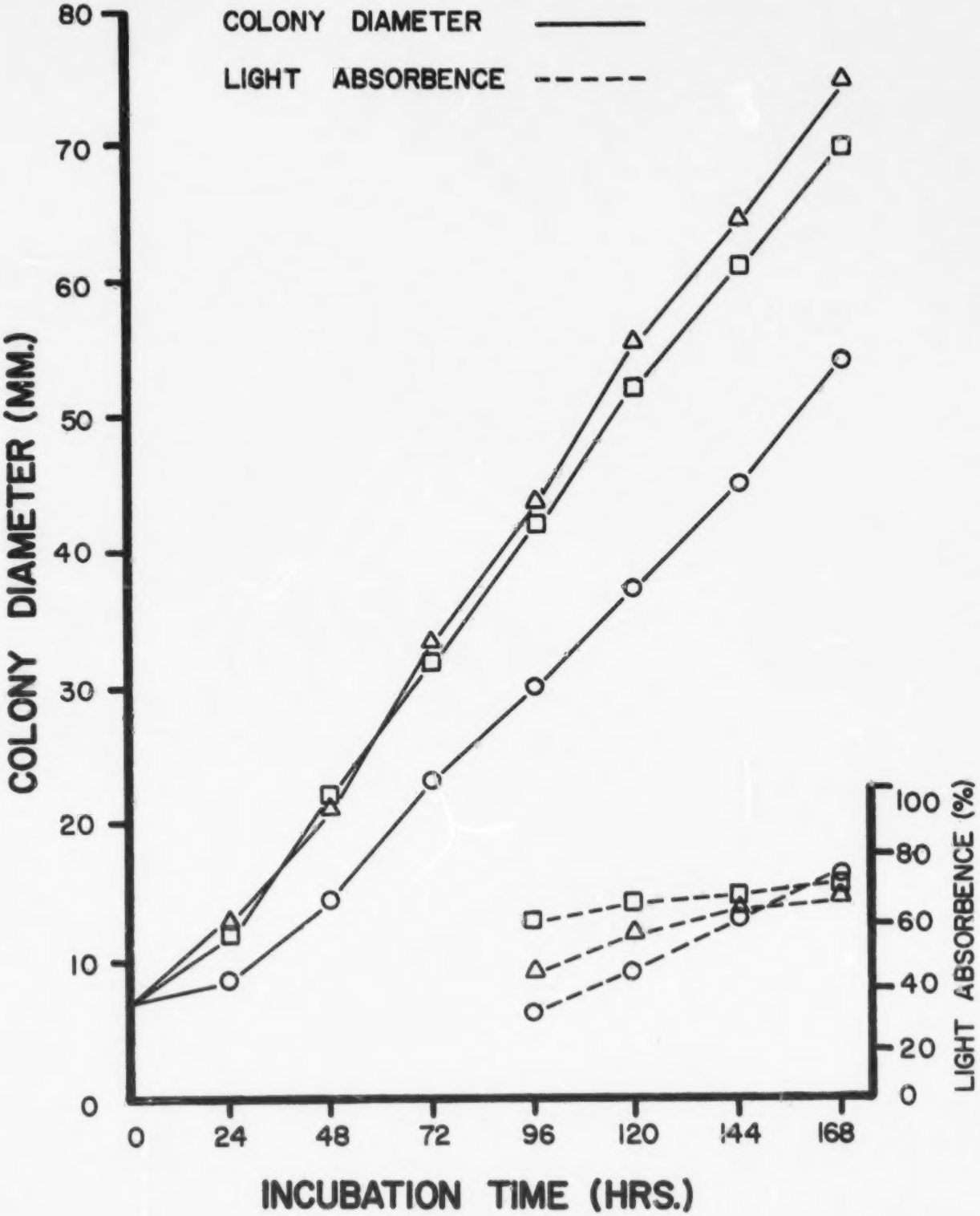
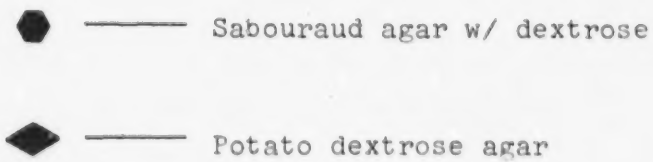

COLONY DIAMETER
LIGHT ABSORBENCE
COLONY DIAMETER (MM.)
LIGHT ABSORBENCE (%)
INCUBATION TIME (HRS.)
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
0
24
48
72
96
120
144
168

FIG. 9. Growth rate studies of Alternaria on commercial media showing colony size and light absorption.



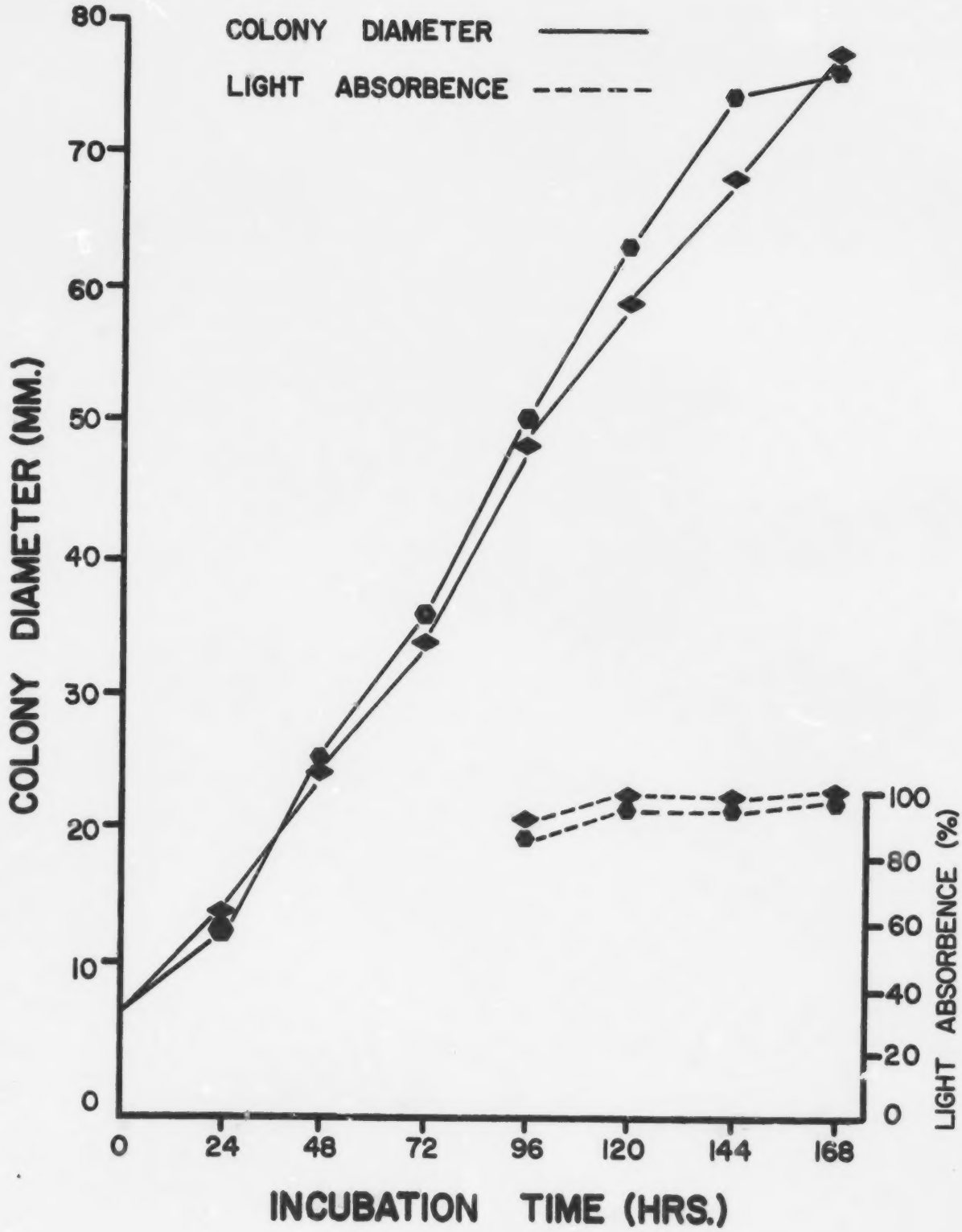
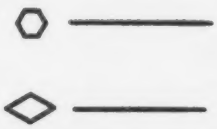

COLONY DIAMETER
LIGHT ABSORBENCE
COLONY DIAMETER (MM.)
0
10
20
30
40
50
60
70
80
0
24
48
72
96
120
144
168
INCUBATION TIME (HRS.)
LIGHT ABSORBENCE (%)
0
20
40
60
80
100

FIG. 10. Growth rate studies of Alternaria on commercial media showing colony size and light absorption.



Malt extract agar

Mycological agar

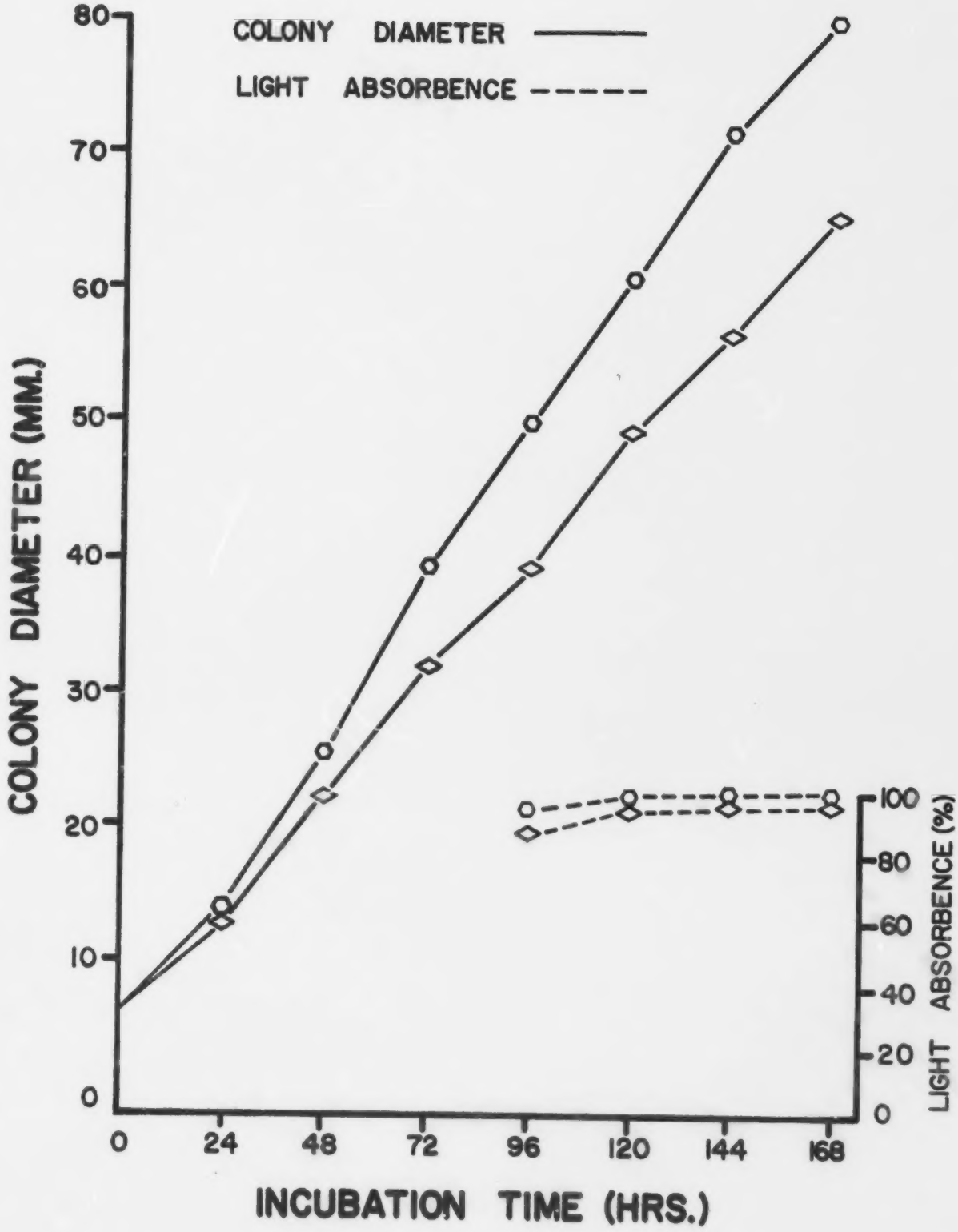

COLONY DIAMETER
LIGHT ABSORBENCE
COLONY DIAMETER (MM.)
LIGHT ABSORBENCE (%)
INCUBATION TIME (HRS.)
0
10
20
30
40
50
60
70
80
0
24
48
72
96
120
144
168
0
20
40
60
80
100

FIG. 11. Growth rate studies of *Alternaria* on unboiled water extract media of *E. crassipes* plant parts.

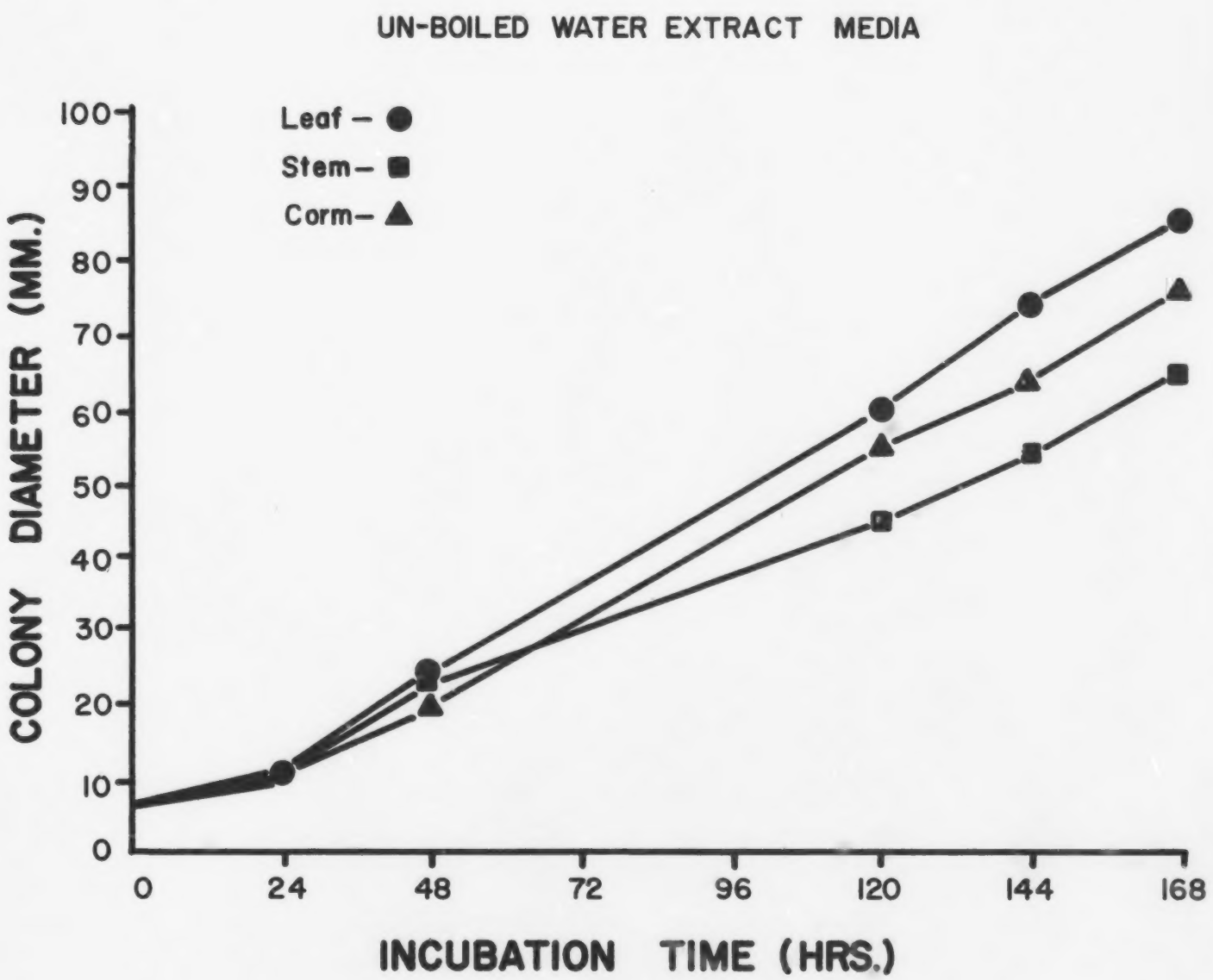

UN-BOILED WATER EXTRACT MEDIA
Leaf –
Stem–
Corm–
COLONY DIAMETER (MM.)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
INCUBATION TIME (HRS.)

FIG. 12. Growth rate studies of Alternaria on unboiled ethanol extract media of E. crassipes plant parts.

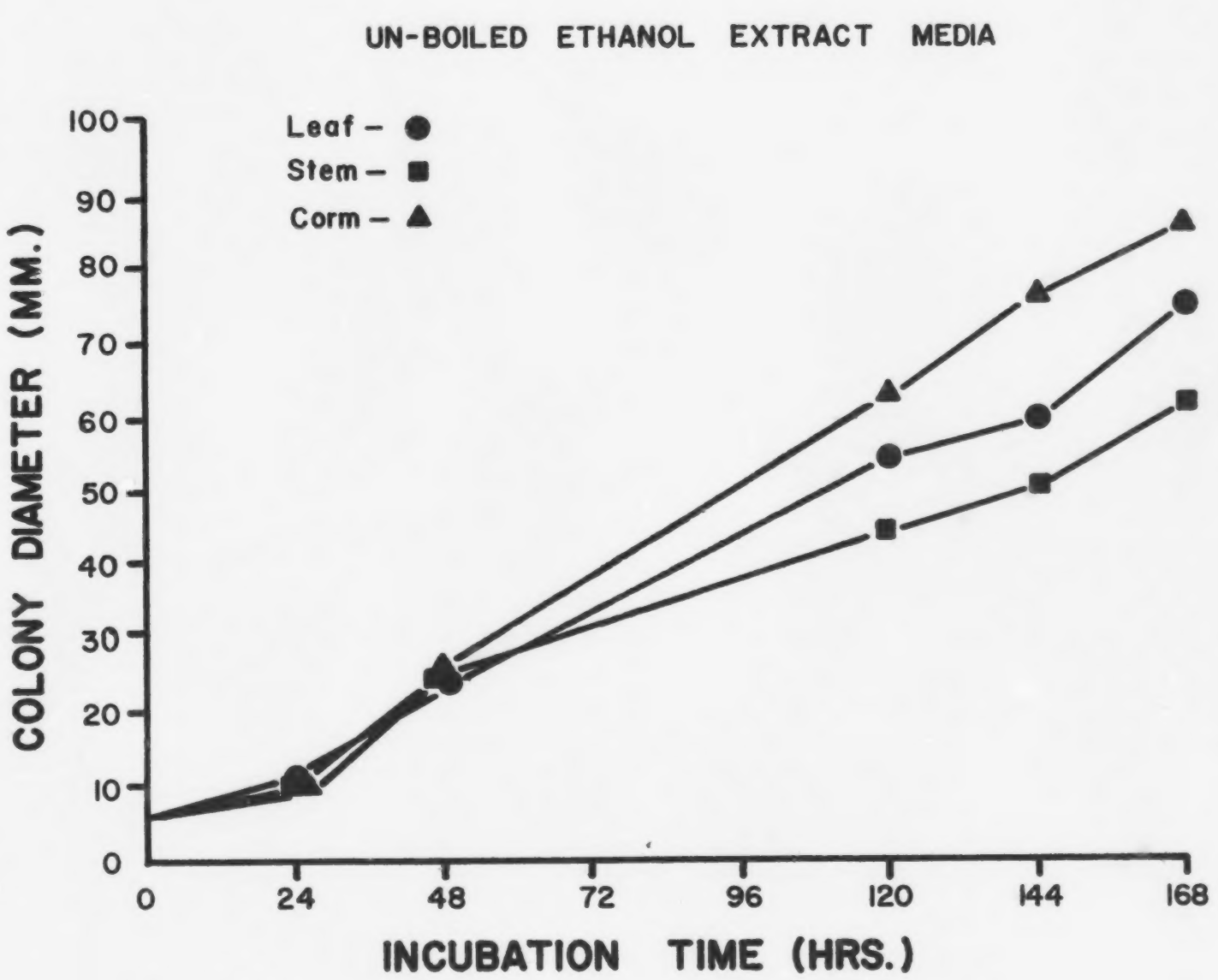

UN-BOILED ETHANOL EXTRACT MEDIA
Leaf –
Stem –
Corm –
COLONY DIAMETER (MM.)
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120
144
168
INCUBATION TIME (HRS.)

FIG. 13. Eichhornia crassipes leaf showing typical leaf spot caused by Alternaria.

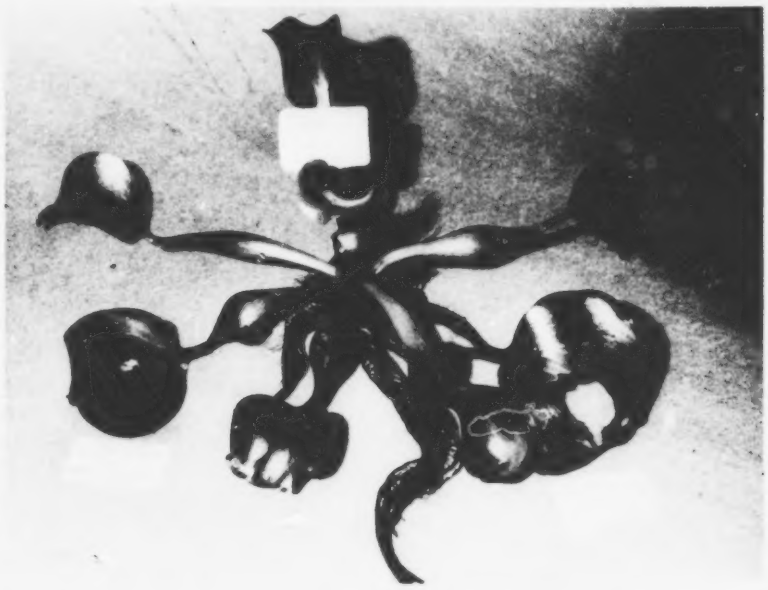


FIG. 14. Eichhornia crassipes plant showing shotting condition as a result of Alternaria inoculation.

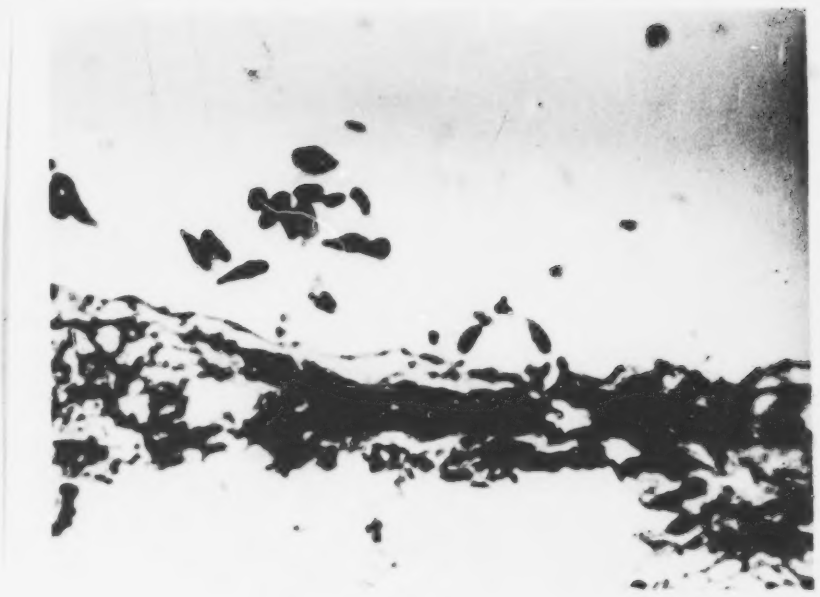
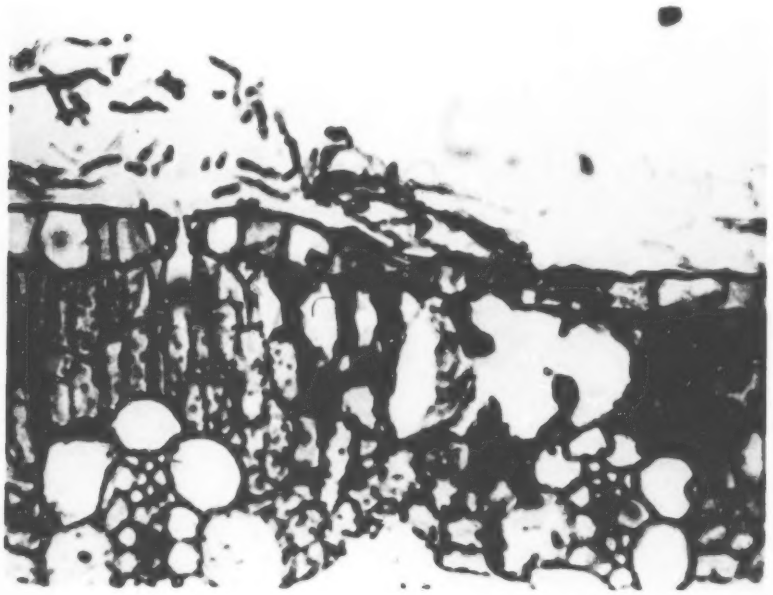


FIG. 15. Serial cross section of leaf spot on E. crassipes leaf caused by Alternaria.

FIG. 16. Serial cross section of leaf spot of E. crassipes leaf showing latter stages of infection.



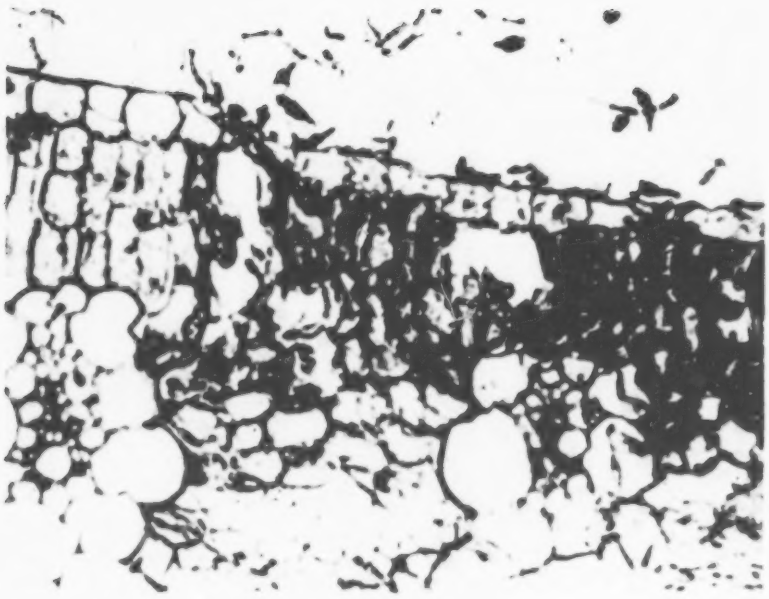


FIG. 17. Serial cross section of leaf spot on E. crassipes leaf showing massive necrotic tissue. Note both inter- and intra cellular hyphae of Alternaria.

FIG. 18. Necrotic tissue on E. crassipes leaf resulting from Alternaria metabolite and area where sterile water was applied.

FIG. 19. Photomicrograph of Alternaria spores. Note chain and branching characteristics. (120X)

FIG. 20. Photomicrograph of Alternaria spores showing classical [illegible]-shaped appearance and septa formation. (100[illegible]

SECTION VIII

DISCUSSION

Our research during the past three years has produced data which is reflective of substantial achievement toward the goal of this project. In a generalized review, the first fiscal year developed some necessary manipulative and cultural techniques of aquatic plant pathology. A survey of south Florida substantiated the existence of infectious morphologies indicative of microbial pathogenesis, i. e., leaf spots, soft rot, yellowing, browning, and others. This was encouraging since our efforts could now be confined to indigenous or previously introduced pathogens, thus alleviating the potentiality of importing exotic pathogens from the plant's native habitat.

Before biological control agents were sought, growth perameters for the host plants were established. Techniques for both isolated laboratory growth and controlled outdoor growth proved valuable in understanding various phenomenom in the field. In the case of Eichhornia in the Bonny Loch study, the results indicate that controlling environmental pressures may be present in some areas of south Florida. Some researchers have indicated that the more effective controlling pressures upon these plants in their native habitat of South America may be those of swiftly moving waters, low light intensities produced by the tropical overgrowth, natural predators, and indigenous diseases. This study presents a further possibility which may exist in south Florida as well as in South America. Oxidizing conditions, positive redox potentials, produced by turbulence and vegetation in South America and by Hydrilla in conjunction with a lack of reducing allochthonous materials in Bonny Loch may oxidize a vital micro- or macro-nutrient in which form the element or compound may be ineffective to the plant. A further possibility exists in that a chemical reaction may occur between the nutrient and calcium carbonate, present in abundance due to the history of Bonny Loch as a limerock excavation. Regardless of the mechanism involved, this study indicates that growth of E. crassipes may be environmentally limited in specific areas of south Florida. Further research is necessary to delineate this mechanism and the limiting substance involved.

In the Lake Wales study involving Hydrilla, several interesting observations were made in the environmental growth chamber studies:

(1) Achlorophic Hydrilla from Lake Wales when grown in reduced light reacquired its normal green color.

(2) When returned to high light intensity these same plants became achlorophic again.

(3) Normal appearing Lake Wales Hydrilla stayed normal looking in the low light; however, became achlorophic in high light.

These observations heavily point to environmental influences being responsible for the apparent disease state of the Lake Wales Hydrilla. The mediating factor appears to be light intensity; however, other factors such as temperature, photoperiod, and nutrients are certainly interrelated to the condition.

Blackburn, et al. (1961) observed that high light intensities on Elodea caused a discoloration of the chlorophylls in leaves, cessation of growth, and death of the plant. It has been reported in many plants that when the intensity of light incident on a photosynthesizing organ is increased beyond a certain point, the cells of that organ become vulnerable to chlorophyll-catalyzed photooxidations. As a result, many more chlorophyll molecules become excited than can be possibly utilized, causing noticeable side effects (Franck and French, 1941). Photooxidation is especially acute in the presence of oxygen resulting in the bleaching of chlorophyll and inactivation of important enzymes. The Warburg Effect, which is the control of photosynthesis by oxygen, has been found to be completely and rapidly reversible when a plant is transferred from an aerobic to an anaerobic atmosphere (Franck and French, 1941).

It should be remembered that various herbicides such as amitrole reduce chlorophyll content, by lowering photosynthetic efficiency of the chloroplasts. Williams and Dunn studied the relationship between diminished chlorophyll content in plants treated with herbicides and color of incident light (Franck and French, 1941). It was determined that red light had the largest effect in reducing chlorophyll content of treated plants. Since Lake Wales has a history of localized herbicide treatment, this factor must be considered in relation to the achlorophic effect.

The research of our second fiscal year resulted in: (1) a didactic comparison of the relative effectiveness of three cidal agents in the surface sterilization of Eichhornia crassipes and Hydrilla verticillata; (2) in vitro plant part cultures; (3) the isolation of a fungal pathogen for E. crassipes; and (4) the establishement of communication through the presentations of reports to others attempting to control aquatic weeds.

In order to isolate organisms from diseased plants, various methods of surface sterilization were developed. Three cidal agents which are routinely utilized in plant pathology for the reduction of surface contamination during isolation techniques are mercuric chloride ($HgCl_2$), sodium hypochlorite (Clorox, NaHClO), and ethyl alcohol. The extension of these agents to aquatic plants for similar use has not been suitably investigated. Since surface sterilization must be accomplished before isolation procedures are valid, studies were initiated to determine the most effective way of eliminating surface contamination from the leaves of the water hyacinth.

Considering the effect of all agents on both fungi and bacteria, ethanol flaming is considered the most effective of the agents investigated when all are employed for the same length of time. Common practice dictates several trials at different times with the selected sterilant upon its utilization for pathogenic isolation. The comparative effectiveness of the agents should be a consideration upon initial selection of the agent.

When selecting a sterilant for use one should also consider the potential for transporting sterilant residue to the isolation medium resulting in selective action on the isolates. Aqueous rinses often suffice for water soluble sterilants; however, with water insoluble agents such as the salts of heavy metals, a toxic residue may remain for which a M/5 sodium thiosulfate rinse is suggested.

Growth of plant parts in aseptic culture was then developed as an aid in pathogenicity studies. The first phase of this research established the optimal culture technique, of those considered, for detached leaf culture of E. crassipes as floatation of the leaves on the surface of media contained in horizontal glass petri plates. This technique was employed during the remaining investigations in this study.

The pretreatment technique using both clorox and Tween 80 solution was successful as a cleansing agent. No apparent damage was inflicted by this treatment. The desired result of clearing the cultures of debris and detritous was attained. According to a comparison between various surface sterilizing agents in association with the isolation of foliar pathogens from E. crassipes, the surface treatment employed for detached leaf culture in this study results in a 10 to 15% reduction in the bacterial and fungal flora on the surface of treated leaves.

Experimental results of the preliminary medium study substantiated the observations of Morrison (1960) that a carbohydrate source enhanced the growth of microorganisms. The media selected as effective from this investigation were benzimidazole and kinetin. Observations of the leaves in culture, however, failed to establish one medium as preferential to the other in the ability to sustain detached leaves of E. crassipes.

Alteration of the light source in the culture of leaves for biochemical analysis from that employed for the other studies was not advantageous. The additional red frequencies of light emitted by the Gro-Lux bulbs and not emitted by the soft white fluorescent tubes encouraged the loss of chlorophyll from portions of the leaves under culture. This occurrence was interpreted as a deleterious influence which accelerated the senescence of the cultured leaves, possibly by providing necessary light for sufficient photosynthesis to exhaust the metabolites stored in the leaves. Therefore, Gro-Lux lights are not recommended for the culture of E. crassipes leaves.

The action of the kinins, kinetin and benzimidazole, has been determined to be upon the chloroplast and the protein synthetic machinery of the cell. This activity has been studied in detail from a biochemical standpoint. Benzimidazole 100 ppm and benzimidazole-kinetin mixture produced a similar stabilizing effect in nitrogen ratios in E. crassipes leaves through two weeks' culture, after which rapid decrease in insoluble nitrogen resulted which caused the ratio of soluble nitrogen to total nitrogen to increase in value (Fig. 5). The rapid rise in ratio exhibited with culture on 50 ppm benzimidazole following one week's culture is considered an aberrant value, a result of the small sample (Fig. 5). If a larger sample were used, the increase to a ratio of 0.78 after three weeks would probably be linear. This reaction is more similar to that of leaves cultured on 50 ppm or the mixture of the kinins. Carbohydrate levels did not reflect the same association, rather those leaves cultured on benzimidazole exhibited a more gradual decline in carbohydrate (Fig. 3) than those leaves on media containing kinetin, including the mixture of the kinins (Fig. 4).

These data indicate that benzimidazole sustains detached E. crassipes more effectively than does kinetin even though only slight differences are apparent in the chlorophyll loss of leaves maintained on these two agents (Table 7). The mechanism of this sustenance on E. crassipes is difficult to establish from the data of this experiment, but a few considerations are pertinent at this point. The gradual depletion of carbohydrate by detached leaves indicates a depletion of energy supplies which will result in a reduced metabolic rate. A reduction in metabolic rate would decrease protein levels due to decreased protein synthesis, resulting in a uniform rate of change in the ratio of protein nitrogen to total nitrogen. This uniform rate of change of leaves treated with kinetin indicates a moderation of the normal metabolism which leads to death of excised leaves. The normal metabolic changes leading to death are represented by the cultures on distilled water. Benzimidazole 100 ppm and the mixture of the kinins did not indicate this gradual change in nitrogen ratios, rather a rapid conversion of insoluble to soluble nitrogen occurred after two weeks' culture. The effect of these two agents upon the metabolism of E. crassipes appears to be an arresting of a degrading process for a period of two weeks, after which the agents are no longer effective. Attainment of a critical level of carbohydrate below which metabolism is curtailed is an effective explanation for the mixture of the kinins, but not for the benzimidazole 100 ppm since the carbohydrate levels of the latter medium are substantially greater at two weeks than that of the former. More detailed biochemical studies are necessary to more fully answer this question.

Regardless of the mechanism involved, metabolism proceeds for two weeks in treated leaves, as indicated by insoluble nitrogen levels, but not longer than seven days on water.

Detached leaves of E. crassipes, therefore, provide an effective substrate for cultivation of pathogens. Obligate parasites are provided the metabolites or synthetic cycles necessary for their replication. The culture of pathogens on detached E. crassipes leaves should, therefore, be as efficacious as the documented pathogenic culture on detached leaves of other plant species.

Provision of a self-sustaining nutrient medium for pathogenic culture is but one aspect of the potential of this system. Detached leaves retain tissue integrity and, therefore, present most of the host's defenses to challenge organisms. This provides a convenient in vitro system for evaluating host-parasite relationships and the screening of primary isolates for pathogenicity. A pathogenic lesion harbors many non-pathogenic agents: saprophytes, environmental contaminants, secondary invaders, and in some instances, symbionts. Upon isolation from the lesion during the search for the causal agent, numerous organisms are frequently isolated, many of which are extraneous non-pathogenic agents. The immediate problem, therefore, is to determine which of the isolates are pathogenic. In documented diseases this problem is minimized by clues which the etiology provides in deducing possible agents which may be sought among the isolates. In dealing with hosts for which disease documentation is scarce, such as E. crassipes, these clues are often unavailable or are inconclusive. All isolates must be "screened" by controlled inoculation of host plants to attempt to reproduce the disease. Detached leaf culture is a convenient system for such screening applications due to the ease with which each host is isolated from other inoculated leaves, the potential for controlled duplication of the test inoculations, the relatively small amount of space occupied by each host as compared to intact plants, and the ease of observation and manipulation of experimental units.

Investigation into the in vitro culture of Eichhornia tissue using several widely used tissue culture media showed no proliferation of undifferentiated cells. Lack of control of intracellular contaminants by antibiotics and fungicides necessitates the use of presently unavailable sterile plants. Suspended culture is suggested as a potentially successful method for growth.

A culture system for detached leaf whorls of Hydrilla utilizing benzimidazole was developed and used for inoculation of suspected pathogens collected from various locations in south Florida. While no pathogen was found, this technique allowed the inoculation of large numbers of suspected pathogens.

This last year has resulted in the isolation of a pathogenic fungus for Eichhornia crassipes. However, a massive amount of work must be done on ways to amplify its pathogenesis, means

of using it for large scale inoculations, and perhaps potentially the most important, its host specificity before this agent could be used as a biological control.

Based on the growth rate studies, if only mycelial growth is needed, then corn meal agar is advisable. If both mycelial and spores are required, then either PDA, mycological agar, V-8 juice agar, or Sabouraud agar w/ dextrose is optimal.

When grown on media containing water extracted plant parts, leaf material was optimum. This stands to reason since Alternaria is a leaf disease indicating a water soluble extract present in the leaves which supports growth. However, ethanol extracted plant parts showed corm material to be the best. This suggests one of two possibilities. One, the ethanol sufficiently denatured the water soluble leaf factor thereby suppressing growth, or, two, there is an ethanol soluble growth stimulator present in the hyacinth corm. Time unfortunately did not permit the characterization of these metabolites and thereby did not show satisfactory conclusion of either case.

The original isolation and description of this particular species of fungus, A. eichhorniae, was done in 1970 at the Commonwealth Institute of Biological Control in India (Nag Raj & Ponnappa, 1970). Based on the host from which it was isolated, chromogenesis in culture, spore size and shape, it was designated as a new species. Critical examination of our isolate revealed close similarities to that of the Indian culture in most categories. There were, however, slight variations which we feel are significant.

For example, when grown on malt extract agar, the Florida isolate produces a yellow-orange, diffusable pigment whereas that produced by the Indian isolate is red. Conidia shap and appearance are also very similar, both appearing in branched and unbranched chains and both being typically club shaped (Fig. 19). There is a slight variation in the size. Those from our culture appear on the average to be 8.0 - 9.0 nm shorter and 4.0 nm wider than those of the Commonwealth's (Fig. 20). In all other instances, the species found in south Florida is in close agreement with the one originally described in India. Since this fungus was subcultured from the host E. crassipes, both here and in India, we feel at this time they are the same species. However, because of the differences in chromogenesis and conidia size, we suggest the Florida isolate to be a different variety and should be recognized as such, namely A. eichhornia var. floradana.

The isolation of this fungus from south Florida's water lends additional encouragement to the concept of biological control for noxious aquatic weeds.

SECTION IX

LITERATURE CITED

1. Blackburn, R. D. 1963. Evaluating herbicides against aquatic weeds. Weeds 11:21-24.

2. Blackburn, R. D., J. M. Lawrence, and D. E. Davis. 1961. Effects of light intensity and quality on the growth of Elodea densa and Heterenthera dubia. Weeds, 9:251-257.

3. Blackburn, R. D. and L. W. Weldon. 1966. Control of elodea in a residential situation. Hya. Control J. 5:36-38.

4. Blackburn, R. D., L. W. Weldon, R. Yeo and T. M. Taylor. 1968. Identification and distribution of certain similar appearing submersed aquatic weeds in Florida. Hya. Control J. 7:

5. Blackburn, R. D., P. F. White and L. W. Weldon. 1968. Ecology of submersed aquatic weeds in South Florida canals. Weed Sci. 16:261-266.

6. Brian, R. C. 1964. The classification of herbicides. In L. J. Audus (ed.) The physiology and biochemistry of herbicides. Academic Press, New York.

7. Browning, J. A. 1954. Breakdown of rust resistance in detached leaves of normally resistant oat varieties. (Abstr.) Phytopathology, 44:483.

8. Browder, L. E. 1964. A modified detached-leaf culture technique for study of cereal rusts. Plant Dis. Reptr. 48:906-908.

9. Cappleman, Jr., L. E. and Thomas T. Sturrock. 1971. Bacteriological Fungal or Viral Control of Aquatic Vegetation. Presentation to 11th Ann. Hyacinth Contr. Soc. Mtg.

10. Chibnall, A. C. 1954. Protein metabolism in rooted runnerbean leaves. New Phytologist, 53:31-37.

11. Chokder, A. H. 1967. Biological Control of Aquatic Vegetation. Ag. Pakistan 18:2, 225-29.

12. Clinton, G. P. and McCormick, F. A. 1927. Rust infection of leaves in petri dishes. Conn. Univ., Storrs Agr. Expt. Sta. Bull. 260.

13. Cross, D. G. 1969. Aquatic weed control using grass carp. J. Fish Biol. 1:27-30.

14. Dean, J. L. 1969. Biology of the crayfish Orconectes causeyi and its use for control of aquatic weeds in trout lakes. Technical paper #24 of the Bureau of Sport Fisheries and Wildlife. Washington, D. C.

15. Ferguson, Frederick F. 1968. Aquatic Weeds and Man's Well-Being. Hyacinth Contr. 7:7-11.

16. Franck, J. and C. S. French. 1941. Photo-oxidation process in plants. J. Gen. PHVS. 25: 309-324.

17. Holm, L. G., L. W. Weldon and R. D. Blackburn. 1969. Aquatic weeds. Sci. 166:699-709.

18. Huffaker, C. B. and L. A. Andres. 1970. Biological Weed Control Using Insects. FAO Int. Conf. on Weed Contr. (In Press).

19. Hyde, R. W. 1969. Method of killing elodea. United States Patent Office, #3,463,628. Washington, D. C.

20. Kaniuka, R. P. (ed). 1968. Biological control of aquatic weeds. The weed eating snail. Agr. Res. 16:8-9.

21. Cell Suspension Cultures of Higher Plants: Isolation and Growth Energetics. Lamport, D.T.A. 1964. Exp. Cell Res. 33: 195-206.

22. Mackenzie, J. W. and L. Hall. 1967. Elodea control in Southeast Florida with diquat. Hya. Control J. 6:37-44.

23. Michewicz, J. E., D. L. Sutton, and R. D. Blackburn. 1972. The White Amur for Aquatic Weed Control. Weed Sci. 20:106-110.

24. Moore, M. B. and Charles R. Olien. Mercury bichloride solution as a surface disinfectant for cereal seeds. Phytopath. 42:471.

25. Morrison, R. M. 1960. Studies of clonal isolates of Erysiphe cichoracearum. Mycologia. 52:388-393.

26. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Toshio Murashige and skoog.

27. NAG RAJ, T. R. and K. M. PONNAPPA. 1970. Blight of water-hyacinth caused by Alternaria eichhorniae sp. nov. Trans. Br. Mycol. Soc. 55:123-130.

28. Penfound, W. T. and T. T. Earle. 1948. The biology of the water hyacinth. Ecol. Mongr. 18:447-72.

29. Person, C., D. J. Samburski, and F. R. Forsyth. 1957. Effect of benzimidazole on detached wheat leaves. Nature, 180: 1294-1295.

30. Preece, T. F. and C. H. Dickinson, Ed. 1971. Ecology of Leaf Surface Micro-organisms. Academic Press, New York.

31. Samburski, D. J., F. R. Forsyth and C. Person. 1958. Metabolic changes in detached wheat leaves floated on benzimidazole and the effect of these changes on rust reaction. Can. J. Botany, 36: 591-601.

32. Seaman, D. E. and W. A. Porterfield. 1964. Control of aquatic weeds by the snail Marisa cornuarietis. Weeds 12:87-92.

33. Sguros, P. L. 1965. Use of manatees for aquatic weed control. Fifth Annual Meeting of the Hyacinth Control Society. Palm Beach, Florida.

34. Sguros, P. L. and T. Monkus. 1965. Observations and techniques in the study of the Florida manatee-Reticent but superb weed control agent. Eighteenth Annual Meeting of Southern Weed Conference. Dallas, Texas.

35. Steward, K. K. 1969. Effects of growth regulators and herbicides on germination of Hydrilla turions. Weed Sci. 17:299-301.

36. Sutton, D. L., L. W. Weldon and R. D. Blackburn. 1970. Effect of diquat on the uptake of copper in aquatic plants. Weed Sci. 18:703-707.

37. Train, R. E., J. A. Busterud and B. E. Willard. 1972. Integrated Pest Management. United States Government Printing Office, #4111-0010, Washington, D. C.

38. Tuite, John. 1969. Plant Pathological Methods: fungi and bacteria. Burgess Pub. Co., Minneapolis.

39. Turel, F. L. M. and G. A. Ledingham. 1957. Production of aerial mycelium and urcduspores by Melampsora lina (Pers.) Lev. 3:813-819.

40. Ward, D. B. 1968. Florida Flora Newsletter #14. University of Florida, Gainesville, Florida.

41. White, W. L. and Sam Frankel. 1965. Seiverd's chemistry for medical technologists. C. V. Mosby Co., St. Louis.

42. Yarwood, C. E. 1946. Detached leaf culture. Bot. Rev. 12: 1-56.

43. Yemm, E. W. and A. J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.

44. Zettler, F. W. and T. E. Freeman. 1972. Plant pathogens as biocontrols of aquatic weeds. Ann Rev. of Phytopath. 10:455-471.